EXPERIMENTAL STUDIES ON THE NATURE OF SPECIES

II. PLANT EVOLUTION THROUGH AMPHIPLOIDY AND AUTOPLOIDY, WITH EXAMPLES FROM THE MADIINAE

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PREFACE

The drama of organic evolution challenges the imagination more strongly than most other topics in the field of biology. This is a particularly difficult process to analyze experimentally for the purpose of gaining an understanding of its mechanism. The myriad elements that enter into it are subject to individual analysis, but a sorting, amalgamation, and comprehension of very numerous experimental data are necessary before some first governing principles can emerge. Genetics, cytology, comparative morphology, and ecology (including distributional and physiological ecology) are among the fields that furnish the critical data. Together, when applied to the study of organic evolution, they comprise *biosystematics.* As yet, biologists are at the very beginnings of an understanding of this vast subject.

In the past score of years great strides have been made toward clarifying perplexing problems in natural relationship. Many aspects of the advances in this field, especially in the animal kingdom, have recently been reviewed by Dobzhansky (1941). In the plant kingdom the patterns of variation are many, but the guiding principles are gradually becoming evident. This progress has come through the accruement and organization of data gathered in a number of related disciplines.

In the present work the objective has been to bring into a new focus the broad picture of species building in plants by amphiploidy and autoploidy. This aspect of plant evolution has received repeated treatment in the past, and the present volume does not attempt to exhaust the subject. Rather, this account aims to present the pattern of organization in nature as disclosed by experimental polyploidy and through the application of biosystematic principles. On the background of their own experiments, the authors venture to assemble much representative material from contemporary literature and examine the evolutionary patterns thereby disclosed.

Division of Plant Biology
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CONTENTS

PAGE

GLOSSARY OF IMPORTANT TERMS vi

CHAPTER

I. INTRODUCTION 1

II. MADIA NUTRAMMII 4

III. MADIA CITRIGRACILIS 22

IV. LAYIA PENTAGLOSSA 46

V. BIOSYSTEMATIC RELATIONSHIPS AND AMPHIPLOIDY 62

VI. CLASSIFICATION OF EXPERIMENTAL POLYPLOIDS ON BIOSYSTEMATIC PRINCIPLES 70

VII. ECOLOGIC CHARACTERISTICS OF NATURAL AMPHIPLOIDS 120

VIII. ECOLOGIC CHARACTERISTICS OF NATURAL AUTOPLOIDS 130

IX. TAXONOMIC AND EVOLUTIONARY ASPECTS OF AMPHIPLOIDY AND AUTOPLOIDY 149

LITERATURE CITED 154

INDEX 165

GLOSSARY OF IMPORTANT TERMS

Terms Describing Relationships between Plants

Ecotype (Turesson, 1922*a*, 1922*b*), all the members of a species that are fitted to survive in a particular kind of environment within the total range of the species (p. 63).

Ecospecies (Turesson, 1922*b*, 1929), all the ecotypes genetically so related that they are able to exchange genes freely without loss of fertility or vigor in the offspring (p. 64).

Cenospecies (Turesson, 1922*b*, 1929), all the ecospecies so related that they may exchange genes among themselves to a limited extent through hybridization (p. 64).

Comparium (Danser, 1929), all the cenospecies between which hybridization is possible either directly or through intermediaries. Gene exchange between cenospecies is impossible (p. 64).

Terms Describing Relations between Chromosome Numbers

Genome (Winkler, 1920), the basic, or haploid, chromosome set of a diploid species.

Ploidy, an addition or multiplication of genomes; ordinarily used with prefixes to signify the degree of duplication, as triploidy, tetraploidy, etc.[1]

Polyploidy (Winkler, 1916), the existence among related organisms of a series of chromosome numbers that are multiples of a basic number, as, for example, $n = 7, 14, 21, 28$, etc.

Dysploidy (Tischler, 1937), the existence among related organisms of a series of chromosome numbers that are not polyploid, as, for example, $n = 5, 6, 7, 9, 11$, etc.

Heteroploidy (Winkler, 1916), the presence among related organisms of chromosome numbers deviating from the diploid. This includes polyploidy, or dysploidy, or both.

Aneuploidy (Täckholm, 1922), hyper- and hypoploidy in an otherwise polyploid series.

Autoploidy (= *autopolyploidy*) (p. 116), the multiplication of the chromosome sets within the limits of one species; "die Verdoppelung desselben Chromosomensatzes" (Kihara and Ono, 1926). Always a form of polyploidy, never of dysploidy.

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[1] Rickett (in Camp and Gilly, 1943, p. 338) contends that "ploid" as used in genetic literature has no true Greek derivation or real significance, because, historically, it was compounded from Weissmann's "id" (*idios*, private), the hypothetical hereditary unit, prefixed by *haplo-* (single) or *diplo-* (double). Nevertheless, later usage has taken up "ploid" from the Greek root *-ploos* (-fold), a combining form, and *-oid* (like). Following this usage, diploid (twofold), tetraploid (fourfold), amphiploid (both-fold), autoploid (self-fold), etc., are etymologically both correct and significant, and haploid could be considered a contracted form of haploploid.

Amphiploidy (= *amphidiploidy*) (p. 117), the addition of all the chromosomes of two distinct species. This is effected through chromosome doubling either before or after hybridization. The term *amphidiploid* was originally applied by Navashin (1927) to a theoretical hybrid of *Crepis* containing two sets of chromosomes of *setosa* ($n = 3$) and two sets of *aspera* ($n = 4$). The parents of amphiploids may have the same or different chromosome numbers, and series of amphiploids may be either polyploid or dysploid.

Alloploidy (= *allopolyploidy*), polyploid amphiploidy (Kihara and Ono, 1926).

I

INTRODUCTION

For more than a decade the writers have been engaged in intensive investigations on the patterns of speciation in the Madiinae, a subtribe of the Compositae. In these studies, taxonomic, ecologic, and cytogenetic viewpoints have been combined in an effort to discover general principles governing the organization of natural plant groups. The Madiinae have proved to be especially well suited for these studies, for the eleven genera and almost ninety species display a wealth of patterns of relationships, and readily lend themselves to experimental study. This subtribe is chiefly confined to California and bordering regions, but has two or three outlying representatives in Chile and neighboring Argentina.

The tempo of species formation appears to vary within wide limits: at one extreme the accretion of gene differences is able to result in the evolution of a new species very gradually; on the other hand, the addition of chromosome sets by amphiploidy can produce a new species precipitately. The latter phenomenon, almost an exclusive feature of the plant kingdom, has been a common method of species formation there. It has usually resulted from the doubling of the chromosome sets in an interspecific hybrid. Another evolutionary process that frequently occurs in plants, less spectacular, but nevertheless important, is the duplication of nonhybrid genomes resulting in autoploidy.

Speciation is accomplished with or without accompanying changes in chromosome number. In plant genera, species more frequently differ by whole sets of chromosomes than by one or two pairs; that is, polyploidy is more common than dysploidy. The subtribe Madiinae is an exception to this rule, for it mostly follows the dysploid pattern. Eighty of its species have been studied cytologically, and all chromosome numbers between 4 and 14 are found, plus 16 and 24. Only six polyploids are known in the wild, five in the genus *Madia* and one in *Layia*. Three Madias and the *Layia* are tetraploid, with $n = 16$ chromosomes, and two Madias are hexaploid, with $n = 24$. From cytological, genetical, and morphological evidence, none of these polyploids are believed to have arisen by simple doubling of the chromosomes of one species (autoploidy). One of the hexaploid Madias has been successfully synthesized by the addition of the chromosomes of two other species, and two more amphiploids, unknown in the wild, have been brought into existence.

These three instances of experimentally produced amphiploids include two cases in *Madia* and one in *Layia*. The first case in *Madia* is of a well balanced, distinctive artificial species unknown in nature, *Madia nutrammii*, $n = 17$, synthesized from *M. nutans* (Greene) Keck, $n = 9$, × *M. Rammii* Greene, $n = 8$. The other is the experimental duplication of the naturally occurring hexaploid species mentioned above, *Madia citrigracilis* Keck, $n = 24$, produced from *M. gracilis* (Sm.) Keck, $n = 16$, × *M. citriodora* Greene, $n = 8$. At the time it was synthesized, this species was thought to be the only naturally occurring hexaploid in the entire subtribe, but during its investigation a closely allied hexaploid form was discovered that is morphologically indistinguishable from tetraploid *gracilis*.

The amphiploid *Layia pentaglossa*, $n = 15$, was produced from the hybrid *L. pentachaeta* A. Gray, $n = 8$, × *L. platyglossa* (F. et M.) A. Gray, $n = 7$. It was genetically less stable than the two amphiploids of *Madia* and did not survive.

The artificial production of these new species has called attention to certain applications of the experimental method in the classification of species and genera and in the exploration of their relationships. The two amphiploids in *Madia* were discovered to be of a different order of stability from that of the one in *Layia*. All three arose through the same cytological mechanism—gametic doubling—but the two successful Madias remained constant, whereas the unsuccessful *Layia* showed interspecific segregation. These facts led to a critical survey of the relationships between the parents, and of the circumstances surrounding the origin of some of the better-studied amphiploids reported in the literature, in the search for fundamental principles that govern the successful production of amphiploids. From this survey it appeared that the success and constancy of amphiploids is linked with the degree of relationship found between their parents.

In this volume the reader will find, following the factual presentation of data concerning the origin of the amphiploids in the Madiinae, a presentation of the authors' views on the application of biosystematic principles to problems of plant relationship and evolution. The natural units are considered in the light of their place in evolutionary succession, and the role of amphiploidy is emphasized. Applying the biosystematic principles, thirty-one of the better-known experimentally produced amphiploids are classified on the basis of the degree of relationship between their parents, and of their behavior after the chromosomes were doubled. Then, in order to gain a perspective on the ecological significance of polyploidy, a number of naturally occurring amphiploids and autoploids are reviewed from this viewpoint and contrasted. Finally, the general importance of amphiploids and autoploids in the evolutionary process is considered.

The authors bear a debt of gratitude to all those workers who have contributed, however modestly, to the field of biosystematics. Without their efforts the facts would not have been available that have proved so essential for the present publication. It is only by attempting to organize the multitude of contributions made by many workers that progress can be made toward improving our view of a subject so vast in scope as that of organic evolution. The topics reviewed in this volume, amphiploidy and autoploidy, are but phases of this subject, yet they are of sufficient importance to impress one with the fundamental similarities in the patterns of relationship governing all higher plants. Once the principles become clear, the stage is set for relatively rapid advances in unfolding the evolutionary patterns in all genera in which amphiploidy and autoploidy occur.

ACKNOWLEDGMENTS

To Miss Marguerite E. Hartung the writers are indebted for her skillful assistance in the preparation of the cytological material. They have also been fortunate in having the benefit of the professional criticism of Drs. E. B. Babcock, R. E. Clausen, and G. L. Stebbins, Jr., of the Division of Genetics, University of California, who kindly read parts of the manuscript.

II

MADIA NUTRAMMII

Madia nutrammii, $n = 17$, was synthesized through the addition of the chromosomes of *Madia nutans* (Greene) Keck, $n = 9$, and *M. Rammii* Greene, $n = 8$. This amphiploid was possibly the most unexpected of the three to be reported here, for until recently *nutans* had been considered to belong to another genus. The experiments and considerations that led to its reclassification as a *Madia* have not previously been reported in full. These facts will therefore be presented before the differences between the parents and the circumstances that led to the production of the amphiploid are considered. The re-evaluation of the taxonomic position of *nutans* will also illustrate one of the experimental approaches to classification.

SYSTEMATIC POSITION OF MADIA NUTANS

In its fifty-year recorded history, *Madia nutans* has been known as a *Layia* or as a member of three other genera now partly or completely merged with *Layia*. During the early stages of the present investigations, it was discovered to be a *Madia*, and was formally reclassified as such (Keck, 1935).

There were good reasons for the uncertainty regarding the generic identity of *nutans*, for in morphological characters it is quite intermediate between *Layia* and the spring-flowering Madias of the *Anisocarpus* section. The ray akenes of *Layia* are compressed dorsoventrally, those of *Madia* laterally, but *nutans* has banana-shaped, and therefore intermediate, akenes. It is a spring-flowering species of delicate habit with rather small heads and scaly pappus, but these characters suggest that it may be a member of either genus. The intricate branching and slender peduncles indicate closer relationship with *Madia* than with *Layia*. The only morphological character definitely linking *nutans* with *Madia* alone is its entire and narrow basal leaves, but this good generic character has generally been neglected by taxonomists because it cannot always be determined on herbarium specimens.

The cytological and genetical investigations made it obvious that *nutans* belongs with *Madia* and not with *Layia*. It has 9 pairs of chromosomes (Johansen, 1933), a number not shared with the Layias, which have $n = 7$, 8, or 16 only. It so happens that one unquestionable species of *Madia* has this same number of chromosomes. This species is *M. Hallii*

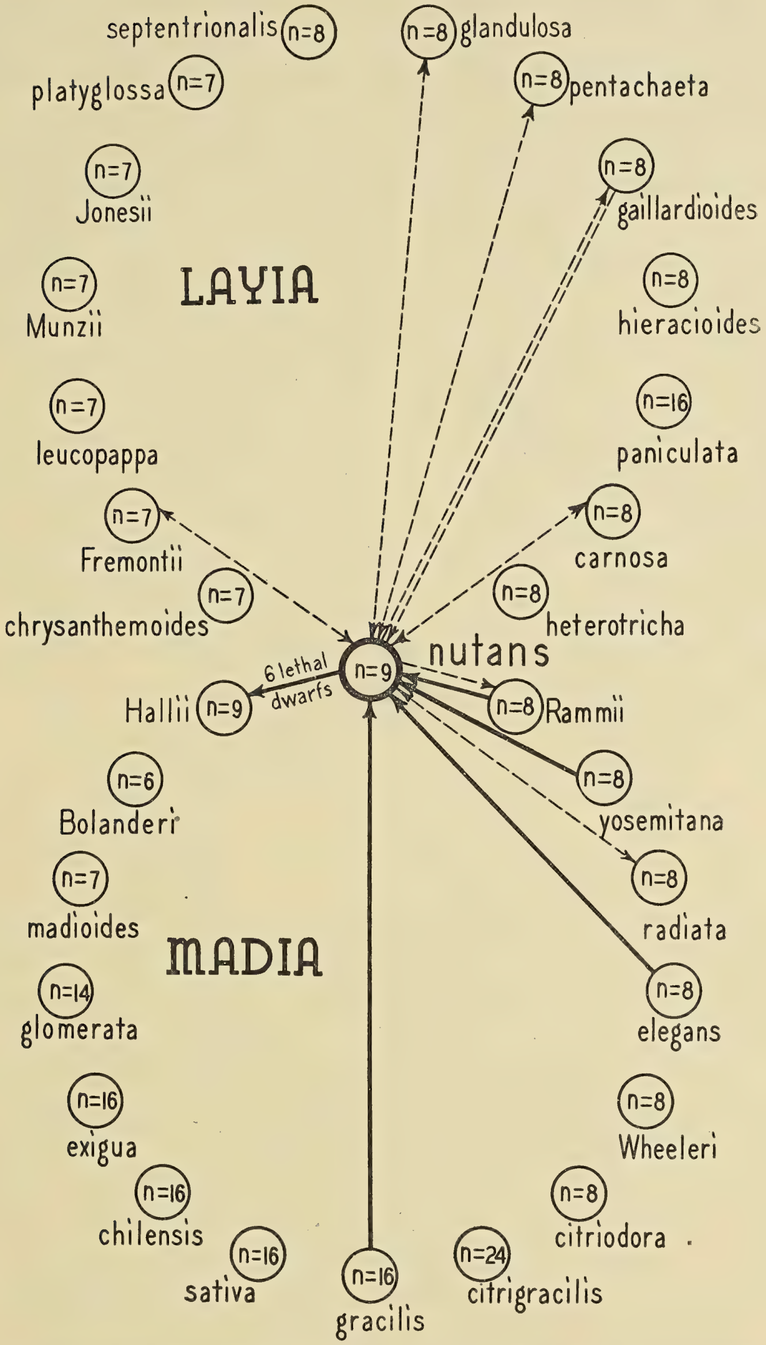


Fig. 1. Diagram of the species of *Layia* and *Madia* with their chromosome numbers. Successful crossings of *Madia nutans* indicated by solid lines; unsuccessful attempts at crossing, by broken lines.

Keck, which also affords a morphological transition between *nutans* and the other Madias.

The genetic evidence is the most conclusive, for *nutans* is unable to cross with any species of *Layia,* but can hybridize with at least five species of *Madia.* The diagram, figure 1, indicates all the species of both genera, their chromosome numbers, and both the successful and the unsuccessful attempts at crossing *nutans* with various species of each.

Actually the test has been broader than figure 1 shows. *Madia nutans* has frequently been grown in an isolation plot with another *Madia* species in the attempt to obtain hybrids through insect pollination. Because *Layia* species were not expected to cross with the Madias, two or more were usually present in the same plot. In spite of the fact that *nutans* thus had the opportunity for crossing with almost all the species of *Layia,* no such hybrids were discovered in cultures of *nutans* grown from this seed.

Also, when *Madia nutans* is permitted open pollination in a garden where it is surrounded by numerous other species and races of *Layia* and *Madia,* it is fertilized only by *Madia* in spite of the preponderance of *Layia* pollen. This is shown by the offspring of 8 plants so pollinated. Of the 50 plants grown from these, the surprising number 33 were first-generation interspecific hybrids with four species of *Madia.* The summation is as follows:

Madia nutans	17 plants
M. nutans × *M. Rammii* F_1	21 plants
M. nutans × *M. elegans* F_1	6 plants
M. nutans × *M. gracilis* F_1	4 plants
M. nutans × *M. yosemitana* F_1	2 plants

In the above instances of uncontrolled hybridization, the parentage of the hybrids could easily be established through morphological characters and verified through chromosome numbers. All the hybrids were completely sterile except those of *nutans* × *Rammii,* which showed the ability to become fertile by doubling the chromosome number and producing a new amphiploid species intersterile with all other Madias. The evidence, then, clearly shows that *nutans* is genetically more closely related to *Madia* than to *Layia.*

SYNTHESIS OF MADIA NUTRAMMII

The parent species. *Madia nutans* and *M. Rammii* belong to the group of small, spring-flowering annuals in the section *Anisocarpus.* Both consist of self-sterile individuals with conspicuous yellow rays. They are rather attractive and much less glandular than the summer- and fall-flowering species of the section *Eumadia,* and lack their ruderal tendencies. On the contrary, both are rare and endemic species, as is shown in the map, figure 2.

Madia nutans grows in the inner North Coast Ranges of California from the Mount St. Helena region southward along the mountains bordering both sides of Napa Valley, at elevations between 150 and 950 meters. It is found on volcanic ash in dry clearings of the chaparral and in forest openings.

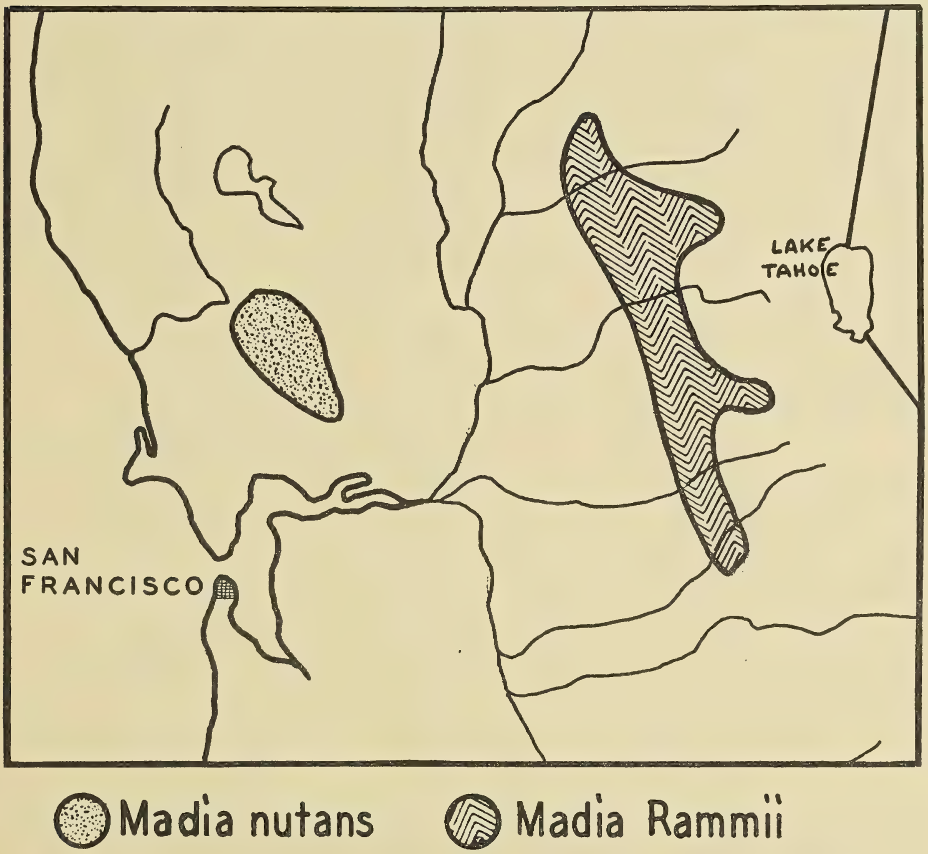


FIG. 2. Distribution of *Madia nutans* and *M. Rammii* in central California

Madia Rammii, which is found in the Sierran foothills from Nevada County to Calaveras County, grows under quite different conditions from *nutans*, in moister, open grassy fields in clayey soils. It is found at elevations between 425 and 1500 meters, although only one station is known above 1000 meters. The arid expanse of the Sacramento Valley effectively bars the two species from coming together in the wild.

About fifty morphological characters separate these species. Nineteen of the more readily distinguishable of these are analyzed in table 1, which also lists the corresponding characters for their synthesized amphiploid descendant, *M. nutrammii.* The differences in habit of growth of the three when grown under greenhouse conditions are illustrated in figure 3.

TABLE 1

MORPHOLOGICAL DIFFERENCES BETWEEN MADIA NUTANS, M. RAMMII, AND THEIR AMPHIPLOID, M. NUTRAMMII

Character	*M. nutans*	*M. nutrammii*	*M. Rammii*
Height	10–25 cm.	20–50 cm.	20–50 cm.
Stems	purple-black	purple	green
Branching	densely paniculate	open-paniculate	subumbellate
Heads	nodding at maturity	slightly nodding	erect
Involucre	turbinate	globose	depressed globose
Ligules	light yellow, broad and short, always open	golden yellow, intermediate, partly open in p.m.	golden yellow, long and narrow, closed in p.m.
Disk florets	7–30	16–41	20–65
Anthers	yellow	black	black
Ray akenes	banana-shaped, subcylindrical, glossy, without pappus	crescent-shaped, laterally compressed, intermediate, with pappus	semicircular, laterally flattened, striate, with pappus
Disk akenes	60% fertile	20–30% fertile	sterile
Disk pappus	10 lanceolate scales, stiff, white	6–9 narrower scales, slightly flexuous, slightly tawny	5 linear awns, flexuous, purple-tipped
Chromosomes	$n = 9$, relatively small	$n = 17$, both kinds	$n = 8$, relatively large

CIRCUMSTANCES OF ORIGIN OF MADIA NUTRAMMII. A total of twenty-four hybrids of the combination *M. nutans* × *M. Rammii* have appeared in our cultures, indicating a fair degree of crossability between these species. None arose from the reciprocal combination. Two of the hybrids were obtained by hand pollination and gave rise to the artificial amphiploid species. Another was produced by insect pollination of one plant of *nutans* surrounded by four plants of *Rammii* in a garden isolation plot. The other twenty-one hybrids occurred spontaneously and are included in the tabulation on page 6.

The two F_1 plants that gave rise to the amphiploid were produced by mutual pollination between one plant of *nutans* from Sarco Creek, in

Napa County, and one of *Rammii* from Grass Valley, in Nevada County, in a screened cage in the greenhouse. Earlier experiments had shown that both species are highly self-sterile, and that mutilation attending emasculation greatly reduces the chances for success in interspecific crosses. Accordingly, emasculation was omitted.

No offspring were obtained from the plant of *M. Rammii,* but 80 akenes harvested on *nutans* produced a population of twelve plants. Two of these were *nutans* × *Rammii* F_1 hybrids, and the remaining ten proved to be selfed individuals of *nutans.*

The production of selfed offspring was rather unexpected because previous artificial self-pollination of hundreds of heads had failed to set seed, but here, in the presence of foreign pollen, ten plants of the maternal type were obtained from six heads. Several authors have reported maternals arising under similar circumstances, but such plants either have been haploids or have been thought to have arisen through parthenogenesis. Two of the ten maternal *nutans* plants were examined and found to have 18 chromosomes in their root tips, showing that they were neither haploids nor hybrids. All were moderately vigorous and did not appear to suffer from inbreeding, and when no pollen of a foreign species was introduced, they remained self-sterile.

Self-pollination in ordinarily self-sterile species, when crossing is attempted with a rather remotely related species, has frequently been observed in the Madiinae. It did not occur when the same plants were emasculated before pollination. This fact and the segregation ratios observed in offspring of the maternal type appear to exclude parthenogenesis as a cause. Evidently the foreign pollen stimulates growth of the plant's own pollen, or keeps the style alive long enough to permit self-pollination. This phenomenon of induced self-pollination does not occur in the Madiinae when races or subspecies of the same or closely related species are crossed, for progeny consisting exclusively of hybrids is then the rule. Induced selfing occurs most frequently when crossings are attempted between members of different species complexes of a genus (i.e., between cenospecies). Consequently, its occurrence is an indication of fairly remote relationship.

The two F_1 hybrids of *nutans* × *Rammii* obtained by hand pollination each had $2n = 17$ chromosomes, namely, 9 from *nutans* and 8 from *Rammii.* Although both hybrids were quite highly sterile, seven F_2 offspring were obtained. The descendants of some of these which became amphiploid are now highly fertile and have been carried through the fifth hybrid generation. A pedigree of these is shown in figure 4. The subnumbered plants listed in this pedigree have all been studied cytologically.

The first-generation hybrids were grown in 1934 and were healthy and vigorous. They resembled one parent in certain characters and the other in others, but were generally intermediate. The analysis of *M. nutrammii* given in table 1 also serves to describe them except as to height, fertility, and chromosome number.

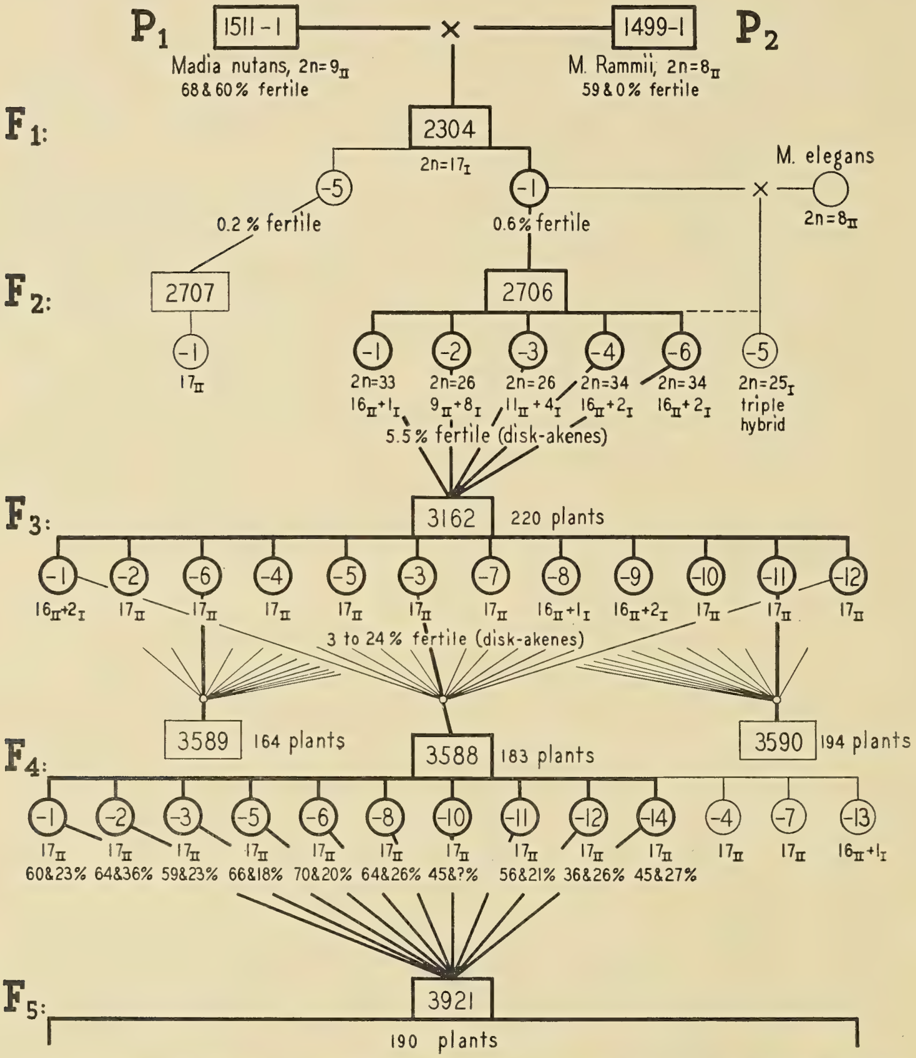


Fig. 4. Pedigree of *Madia nutrammii*, showing parentage, number of plants, chromosome numbers, pairing, and fertilities. (Where two fertilities are given for one plant, the first refers to that of ray florets, the second to disks.)

At the time when these hybrids were obtained, no offspring was expected from mutual pollination of the two small F_1 plants because of the very remote relationship of their parents. One F_1 hybrid was therefore permitted to pollinate openly in an unscreened greenhouse, where it could backcross with either parent or outcross with several races of *Madia elegans* D. Don. This plant is 2304-1 of figure 4. The other (2304-5) was open-pollinated in the garden, where pollen of the parental species, of *M. elegans*, and of many other species was abundantly available. Some 1000 akenes were harvested on the greenhouse plant, of which 28 appeared to be good, and approximately 500 on the field plant, of which only 5 appeared to be viable. All were sown; six offspring were obtained from the greenhouse plant, and one from the hybrid in the garden. The fertilities of the F_1 plants, therefore, were well below 1 per cent.

Six of the seven second-generation plants were essentially alike and similar to the F_1 hybrids. The seventh (2706-5 of fig. 4) was a triple hybrid, *Madia* (*nutans* × *Rammii*) × *elegans*, with $2n = 25$. Of the others, three had $2n = 34$, one $2n = 33$, and two $2n = 26$ chromosomes. Eleven of the fourteen gametes that gave rise to the seven plants were therefore diploid or nearly diploid, containing both the 9-chromosome genome of *nutans* and the 8-chromosome one of *Rammii.* Accordingly, at least four of the six true F_2 plants had arisen through self-pollination of the F_1 individuals, since these were separated. The selfing may have been stimulated by the presence of pollen of other species.

From this it is evident that practically the only viable gametes of the F_1 were those that contained 17 chromosomes, the full complement of both the parental species. This includes the ovule ($n = 17$) fertilized by *Madia elegans* ($n = 8$) which gave rise to the triple hybrid *M.* (*nutans* × *Rammii*) × *elegans* ($2n = 25$). It was a triple hybrid in the strict sense that complete chromosome sets of three species were added together, rather than merely a recombined F_1 hybrid complement added to that of a third species. This plant inherited characters from all three parents, but those of *elegans*, which was the largest of the three, were most influential. In the conspicuous characters of foliage, pubescence, length of central leader, size and shape of heads and ligules, and ray akenes, this hybrid reflected *elegans*, but its paleaceous pappus was inherited from *nutans*, and its involucral bracts from *Rammii.* The characters of *Rammii* were rather submerged, probably because it differs from *nutans* in the same general direction as does *elegans*, but to a lesser degree. No chromosomal pairing was found in this hybrid, an indication that the three parental species are but remotely related; and no viable akenes were obtained.

In order to obtain seed for an F_3 generation, the five F_2 plants indicated in figure 4 were isolated for mutual pollination in a plot 400 meters removed from all other Madiinae. Three of the five were tetraploid or near-tetraploid plants and two were triploids. Their average fertility was 5.5 per cent, an improvement over the 0.6 per cent of the F_1 hybrid.

Part of the seed harvested on the five F_2 plants, when sown, yielded an F_3 of 220 remarkably uniform plants similar in appearance to the F_1. There was some variation in size, but no more than in pure cultures of the parental species. A sample of twelve plants (3162-1 to -12, fig. 4) was studied cytologically. Of these, eleven had the full amphiploid chromosome complement ($2n = 34$), and the twelfth had one chromosome less. This marked tendency for all the F_3 plants to be amphiploid indicates that a strong selection of F_2 gametes must have taken place, particularly in the two triploid individuals.

The twelve F_3 plants of which the chromosome number was determined were grown in an isolation plot during the summer of 1936 for mutual pollination, and the seed of each was harvested separately. The fertility of the disk akenes varied between 3 and 24 per cent and averaged 11.8, which marked a considerable improvement over the second generation. In this connection it should be remembered that one of the parent species, *M. Rammii*, has sterile disk akenes.

Part of the akenes from three of the most fertile and cytologically regular F_3 plants in the isolation plot were sown in 1940, and the resulting F_4 cultures (3588, 3589, and 3590 of fig. 4) totaled 541 plants. These three cultures were alike, vigorous, and as uniform in their characters as the members of either of the parent species. They retained the characteristics of the F_1 and were unlike any other species. The chromosome number was determined in a sample of 13 plants. In the root tips of 12 of these the diploid number was 34 (regular amphiploid), and in 1 plant it was 33.

Full fertility was attained by the new amphiploid species in the fourth generation. As is indicated in table 1, *Madia nutans* has fertile disk akenes, and *Rammii* has sterile ones. Both species are approximately equally fertile with respect to ray akenes (68 and 59 per cent, respectively). Since the disk akenes of *nutans* are only about 60 per cent fertile, it is obvious that when that species is combined with one with sterile disk akenes, complete fertility can hardly be anticipated in the disk akenes of their amphiploid hybrid. Actually, these fertilities in the thirteen F_4 plants tested ranged between 18 and 36 per cent, with a mean of 24.4 per cent. Likewise, the fertilities of the ray akenes averaged 56.5 per cent, which is within the range of fertility of the parental species. The fertilities of each F_4 plant are indicated in figure 4. Two values are given, the first representing the fertility of the ray akenes in per cent, and the second, the fertility of the disk akenes. These values are readily compared with those given for the parent plants of *nutans* and *Rammii*.

An F_5 generation, 3921 in figure 4, consisting of 190 plants, was grown from a small part of the seed harvested on 3588 after open pollination in the regular garden plot, where the plants were exposed to pollination from different strains of the parental species and also many other Madias and Layias. This F_5 maintained the striking uniformity of the preceding generations, and no cases of outcrossing were found.

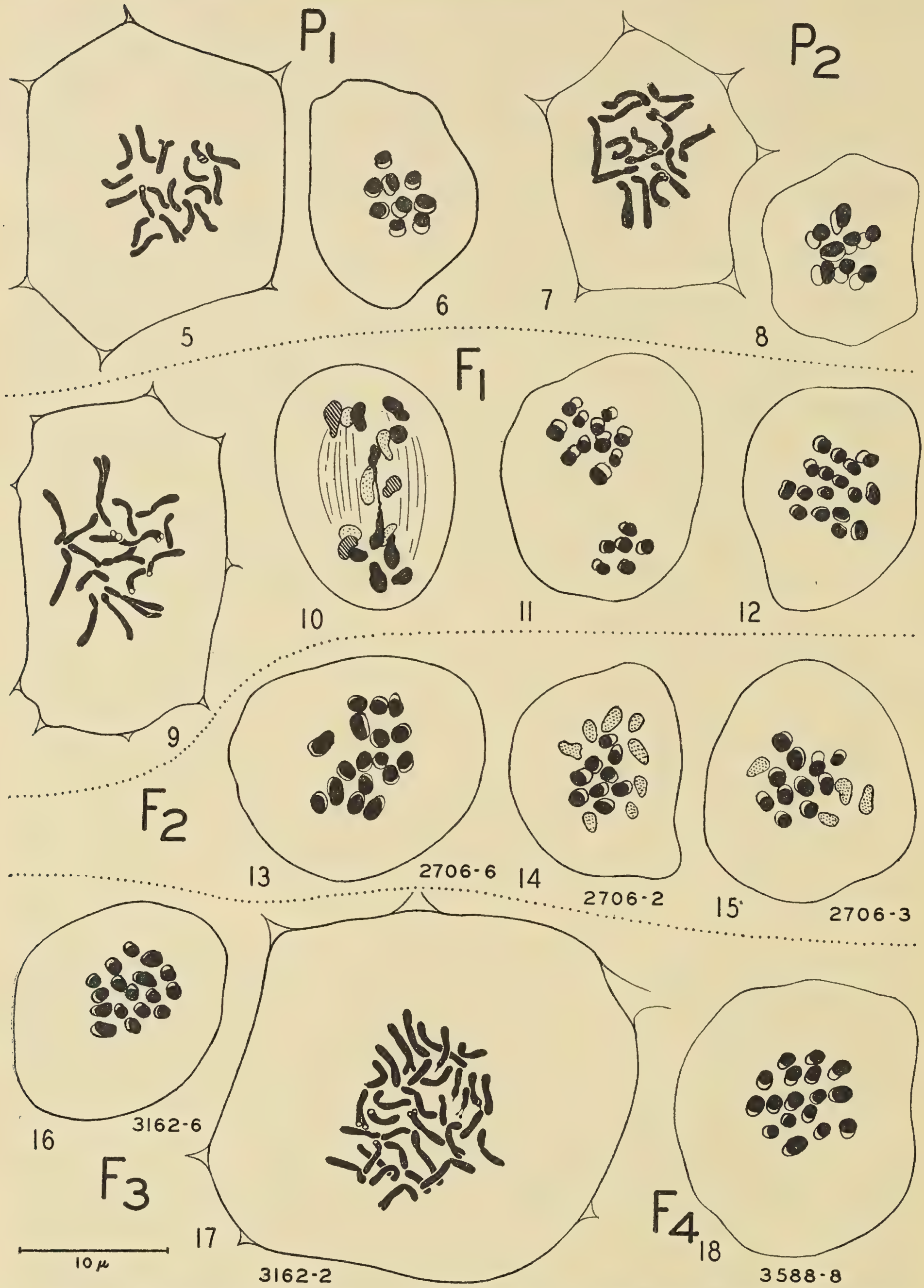
CYTOLOGY OF THE PARENT SPECIES AND THE F_1. *Madia nutans* and *M. Rammii* exhibit great regularity in pairing and distribution of chromosomes in pollen mother cells during meiosis. The chromosomes of *Rammii* are in general somewhat larger than those of *nutans*, but different pairs in each species vary in size. This difference in size can be seen by comparing camera lucida drawings of both somatic and meiotic chromosome plates made at the same magnification, as shown in figures 5 to 8. It is not possible to identify the chromosomes of each species in the F_1 hybrids and in the subsequent amphiploid, but one can conclude with considerable assurance that the largest chromosomes are derived from *Rammii*, and the smallest from *nutans*.

Three F_1 plants were thoroughly investigated with respect to the behavior of the chromosomes during the first and second meiotic divisions of pollen mother cells. These included the two plants, 2304-1 and -5, indicated in figure 4, and another plant which arose as a spontaneous hybrid from open pollination in the garden. Still a fourth F_1 plant that arose like the last was studied in mitosis, but suitable meiotic stages were not observed. Micropollen was a characteristic feature of this plant. Root-tip plates of all four of these F_1 plants had $2n = 17$ chromosomes (see fig. 9).

Meiosis was highly irregular, with very little pairing. The few pairs that were formed (0 to 3) were loosely conjugated with never more than a single chiasma. Too few pairs were present to form a well defined equatorial plate at metaphase. A typical side view of a cell in first metaphase is shown in figure 10, with one loose pair and 15 univalents scattered over the spindle. Frequently the pairs were observed to be heteromorphic, one partner being larger than the other. This condition might be anticipated from the difference in size between the chromosomes of *Rammii* and *nutans*.

The results from a study of 75 intact cells in first metaphase of the two F_1 plants, 2304-1 and -5, are presented in the left half of table 2. The low average of 1.55 pairs per cell is an indication of very remote chromosomal relationship between the parental species. A similar study of 31 cells of a third F_1 plant gave essentially the same results.

When pairing is as weak as in the present case, a very irregular mode



Figs. 5–18. Somatic and meiotic chromosome plates of *Madia nutans* (P_1), *M. Rammii* (P_2), the F_1, and derivatives, including *M. nutrammii* (figs. 13, 16–18). Figure 12 is of a cell which would lead to diploid pollen; it and figure 11 are of adjacent cells in second metaphase. In figures 14 and 15 the unpaired chromosomes are stippled. Plant numbers are indicated from the F_2 to the F_4 (cf. pedigree, fig. 4). × 2000.

of distribution of the chromosomes to the daughter nuclei is to be expected, and this was actually found. Although a distribution of 8 chromosomes to one pole and 9 to the other was fairly common, more irregular distribution was often observed (fig. 11). This information is summarized in the right half of table 2, where the observed distribution of chromosomes in second metaphase is recorded for 27 intact cells.

TABLE 2

Behavior of chromosomes in meiosis of two F_1 plants of Madia nutans, $n = 9$, × M. Rammii, $n = 8$

Pairing, metaphase I	Number of cells	Distribution, metaphase II	Number of cells
17_I	9	16 + 1	1
$1_{II} + 15_I$	29	13 + 4	1
$2_{II} + 13_I$	25	12 + 5	3
$3_{II} + 11_I$	12	11 + 6	6
		10 + 7	10
Total cells	75	9 + 8	6
Mean pairs	1.55		
		Total cells	27

In some cells not all the chromosomes were included in the daughter nuclei, but lagging chromosomes were observed in both first and second meiotic anaphases. These led to the formation of micronuclei, asymmetrical tetrads, pentads, hexads, and even octads. The pollen was of very unequal size, and many of the grains degenerated in early stages, leaving empty cells.

The chromosome numbers of the offspring of the two F_1 plants, namely, six F_2 plants and one triple hybrid, show that 11 out of 13 F_1 gametes were diploid. The random type of distribution shown in the right half of table 2 is not sufficient to explain this high frequency of diploid gametes. Another mode of division was found, however, which leads directly to the production of such cells. Some pollen mother cells appeared to be in first metaphase but had 17 "pairs" of chromosomes regularly aligned in an equatorial plate, instead of slight pairing and scattered singles. Such cells occurred interspersed between others clearly in second metaphase. Figure 11 shows a cell in second metaphase with a 6 + 11 distribution to the daughter nuclei. This was in the same anther lobe with the cell shown in figure 12, which has 17 dividing chromosomes all in one plate. There is therefore little doubt that what appears to be first metaphase actually corresponds to the second.

Such a situation could arise in cells in which the chromosomes failed to separate during the first division, or in which the daughter nuclei remained so close together that they fused again upon entering the second meiotic division. The presence of a number of dyads among the tetrads and pentads supports the conclusion that only one division took place. The cells of the dyads were larger than those of the tetrads and presumably produced the largest pollen.

Diploid gametes were found more frequently in certain anther lobes. In one such lobe there were 11, together with a cell in second metaphase showing a random 8 + 9 distribution. Such lobes presented a marked cytological contrast with those in which random distribution only was found. Probably highly localized physiological conditions favor one mode of division or the other in such a remote hybrid, for the tapetal cells adjacent to pollen mother cells producing diploid gametes have been observed to be especially large and well developed.

The diploid sex cells went through their equational division with marked regularity, although occasionally one or two chromosomes might split precociously. Out of a total of 96 intact pollen mother cells studied in two F_1 plants, 20 were of the type leading to the production of diploid pollen. This represents 20.8 per cent of the total, but this figure may be higher than a true statistical value, because such cells were the object of special search.

The mechanism for diploid gamete formation as described above is similar to that investigated by Rosenberg (1917, 1927) in certain apomictic species of *Hieracium.* He described the phenomenon as a "semiheterotypic division" resulting in a "restitution nucleus" and dyad formation.

CYTOLOGY OF THE F_2 GENERATION. It will be recalled that six offspring were obtained from the F_1 hybrid open-pollinated in the unscreened greenhouse, and one from the second F_1 individual open-pollinated in the garden. These progeny could have arisen in any one of three ways, namely, (1) through self-pollination induced by the presence of foreign pollen, (2) through backcrossing with either *nutans* or *Rammii*, or (3) through crossing with a third species to form a triple hybrid. Actually, three of the seven plants were amphiploids with $2n = 34$ chromosomes, one was nearly amphiploid with $2n = 33$, two were triploids with $2n = 26$, and one was a triple hybrid with *Madia elegans*, with $2n = 25$. The latter plant has already been discussed, on p. 11.

Judging from their chromosome number and morphological characters, the four tetraploid or near-tetraploid F_2 plants could only have arisen through self-pollination of the F_1 by the union of diploid or near-diploid gametes. The 33-chromosome plant (2706-1 of fig. 4) did not differ in appearance from the three straight tetraploids.

The three 34-chromosome plants exhibited minor irregularities in chromosomal behavior during meiosis, such as the occasional lack of pairing of a few chromosomes, lagging of univalents during first and second anaphase, irregular alignment or clumping together of pairs, and stray chromosomes during second metaphase. However, in even the most irregular 34-chromosome plant (2706-4 in fig. 4), about 80 per cent of the cells were regular during first metaphase, and there was no more than 5 to 10 per cent dwarf pollen. The most regular tetraploid was 2707-1, but 2706-6 also often had 17 pairs, as is shown in figure 13. The 33-chromosome plant (2706-1) was more irregular, especially in certain anther lobes in which pairing appeared to break down, although in other lobes $16_{\mathrm{II}} + 1_{\mathrm{I}}$ was the regular pattern.

Although the two triploid plants in the F_2 populations (2706-2 and -3 of fig. 4) each had $2n = 26$ chromosomes, their meiotic behavior was different, indicating different gametic origins. Plant 2706-2 generally had 9 pairs and 8 singles during first metaphase, as is seen in figure 14. Presumably, therefore, the 8 singles consisted of a complete set of *Rammii* chromosomes, and the 9 pairs of two full sets of *nutans* chromosomes. This conclusion is supported by the fact that there are some large *Rammii*-like chromosomes among the singles. The very broad pappus also suggested a preponderance of *nutans* chromosomes. It is therefore concluded that this triploid arose through the fusion of a diploid gamete from the F_1, containing full sets of *nutans* and *Rammii* chromosomes, with a haploid gamete containing only *nutans* chromosomes. This plant could have arisen through induced self-pollination, or, more likely, through a backcross with *M. nutans.*

The other triploid, 2706-3, generally produced 11 pairs and 4 singles at meiosis (the number of pairs varied from 9 to 13). This situation suggests that this plant probably arose from self-pollination of the F_1, and that in rare instances sex cells containing only partial sets of chromosomes of the different species can function and produce a healthy individual. Evidently, however, its sterility was so high that this particular combination of chromosomes was not transmitted to the next generation.

CYTOLOGICAL OBSERVATIONS ON THE F_3 AND F_4. The rather irregular cytological pattern of the F_2 generation was replaced by a much more regular one in the F_3. In this generation the triploids had been eliminated by natural selection, and the meiotic mechanism had materially improved. The chromosomes were more widely separated, better aligned, and better timed during first metaphase. By the fourth generation these improvements were even more pronounced.

Figure 16 shows regular meiotic pairing in first metaphase of one F_3

plant, and figure 17 a somatic plate from a root tip of another. Figure 18 shows a typical first-metaphase plate of an F_4 plant. All these plants have 34 chromosomes.

Some irregularities were found, however, as is shown by table 3. Here are summarized the results of a cytological study of twelve plants of the F_3 (3162) and thirteen plants of the F_4 (3588). Both root tips and buds were examined in all these. It will be noticed that two of the chromosomes occasionally fail to pair in most of the plants of both generations. The same situation was found in the 33-chromosome plants, except that

TABLE 3

PAIRING OF CHROMOSOMES AND FERTILITY OF DISK AKENES IN F_3 AND F_4 OF MADIA NUTRAMMII

Plant number	$2n$	Usual pairing	Comments	Fertility (per cent)
THIRD GENERATION				
3162-1.......	33 + f	$16_{II} + 1_{I} + f$	Fragment equal to half chromosome	20
-2.......	34	17_{II}	Rarely $16_{II} + 2_{I}$	9
-3.......	34	17_{II}	Rarely $16_{II} + 2_{I}$	16
-4.......	34	17_{II}	Often $16_{II} + 2_{I}$	13
-5.......	34	17_{II}	Regular	9
-6.......	34	17_{II}	Regular	24
-7.......	34	17_{II}	Rarely $16_{II} + 2_{I}$	9
-8.......	33	$16_{II} + 1_{I}$	Rarely $15_{II} + 3_{I}$	7
-9.......	34	$16_{II} + 2_{I}$	Often 17_{II}	3
-10......	34	17_{II}	Regular	6
-11......	34	17_{II}	Regular	16
-12......	34	17_{II}	Rarely $16_{II} + 2_{I}$	10
FOURTH GENERATION				
3588-1.......	34	17_{II}	Regular	23
-2.......	34	17_{II}	Rarely $16_{II} + 2_{I}$	36
-3.......	34	17_{II}	Rarely $16_{II} + 2_{I}$	23
-4.......	34	17_{II}	Rarely $16_{II} + 2_{I}$	..
-5.......	34	17_{II}	Rarely $16_{II} + 2_{I}$	18
-6.......	34	17_{II}	Rarely $16_{II} + 2_{I}$	20
-7.......	34	17_{II}	Rarely $16_{II} + 2_{I}$	..
-8.......	34	17_{II}	Rarely $16_{II} + 2_{I}$	26
-10......	34	17_{II}	Rarely $16_{II} + 2_{I}$	..
-11......	34	17_{II}	Rarely $16_{II} + 2_{I}$	21
-12......	34	17_{II}	Often $16_{II} + 2_{I}$	26
-13......	33	$16_{II} + 1_{I}$	Rarely $15_{II} + 3_{I}$	..
-14......	34	17_{II}	Rarely $16_{II} + 2_{I}$	27

no more than 16 pairs were possible. These minor cytological irregularities led to losses of chromosomes and the formation of micropollen. Such irregularities were not found in the parental species, and are probably attributable to slight maladjustments that may eventually disappear in later generations.

Table 3 also affords opportunity for a comparison between meiotic irregularity and fertility. The fertility of the disk akenes is used as an index of fertility in general. Two facts stand out in this comparison. First, there is the rather uniform increase in fertility in the F_4. The third generation contained some plants that were as fertile as those of the fourth, but its fertility was still very uneven. The second fact is the lack of correlation between fertility and pairing of chromosomes. Some of the most regular F_3 plants had low fertility, but the cytologically irregular plant 3162-1, which had a fragmented chromosome, was among the most fertile. Irregularities like those observed in F_3 and F_4 do not seem to impair materially the fertility of the new species.

REPETITION OF THE SYNTHESIS OF MADIA NUTRAMMII. The circumstances that led to the origin of *Madia nutrammii* indicate that the genomes of its parental species, when added together, interact to produce a new successful genetic balance, resulting in a distinctive and true-breeding new species. It should therefore be possible to resynthesize *nutrammii* if this assumption is correct. This has been accomplished from hybrids probably derived from different strains of *Rammii.*

Five of the spontaneous F_1 hybrids of *nutans* × *Rammii* listed in the tabulation on page 6 were isolated in a plot for mutual pollination in 1935. The *nutans* parent of these hybrids was of the Sarco Creek strain, which gave rise also to the artificially produced hybrid, but the pollen could have come from any one of three strains of *Rammii,* originating within a distance of 40 miles in the wild. These strains were quite similar in appearance, although one was larger and more robust than the other two. It is therefore likely that the five F_1 plants selected for propagation were pollinated by different parents.

In their morphological characters the spontaneous hybrids corresponded to the ones produced in the cage crossing. Their fertility was also similar, for from five F_1 plants only 38 apparently good akenes were obtained, 5 of which germinated, and only three F_2 plants survived. These three plants corresponded closely to *Madia nutrammii* both in morphology and in cytology, for they were either amphiploid or near amphiploid. Two plants had 17 pairs of chromosomes and were cytologically as regular as the F_2 plant, 2707-1, and later generations of the new species. The third plant had 31 chromosomes in its root-tip cells, with 14 pairs and 3 singles in meiosis. It was therefore hypotetraploid. Accordingly, five of the six sex cells that produced this F_2 generation were diploid, and the sixth was near diploid.

MADIA NUTRAMMII AS A SPECIES

Madia nutrammii fulfills the requirements of a good species because: (1) it differs from all other species by characters of specific magnitude in the genus; (2) it reproduces only its own kind; and (3) it is genetically isolated from all other species. It also contains within itself a certain amount of individual variation, such as is typically found in natural populations.

Madia nutrammii extends the long series of dysploid chromosome numbers in the Madiinae, for it is the only 17-chromosome species in that subtribe. This demonstrates that dysploid as well as polyploid series of chromosome numbers may arise through amphiploidy. When Winge (1917) suggested that new chromosome numbers evolve by addition of the chromosomes of pre-existing species, the theory was used to explain the origin of the polyploid series only, and succeeding authors have continued to emphasize this point alone.

The new species arose from a single individual of a hybrid between two distinct cenospecies. No special effort was made to produce an amphiploid, except to isolate a small hybrid F_2 population of self-sterile plants that varied in their number of chromosomes. Natural selection did the rest.

The specifically different chromosomes of this hybrid were nonhomologous and did not conjugate, but diploid gametes were occasionally produced which combined to synthesize the amphiploid in the second and third generations. The new species emerged gradually: by the third generation it was cytologically essentially regular, and by the fourth, it had attained the fertility of the parental species.

In the production of *Madia nutrammii* it was observed that almost the only viable sex cells were those which combined the genomes of the parental species. The 9 + 9 *nutans* chromosomes, added to the 8 + 8 *Rammii* chromosomes, made possible normal pairing and produced a new successful genetic balance. This combination was resynthesized when *nutans* and *Rammii* chromosomes from different sources were brought together. Many random assortments of parental chromosomes observed in the sex cells of the F_1 did not survive to produce successful offspring.

It is not yet known whether *Madia nutrammii* is fitted to survive in any existing natural habitat. Its increase in chromosome number did not adapt it for a higher altitude than that in which its parents grow, for when it was sown at the Mather transplant station in the Sierra Nevada, at 1400 meters elevation, it did not succeed. It did not germinate with the other spring annuals in that environment, but appeared early in July after the ground had become considerably warmer. From then on it developed very slowly, producing only dwarf plants with few flowers. In this reaction it behaves like the species native only to the Coast Ranges described in a previous publication (Clausen, Keck, and Hiesey, 1940). It thrives in the garden at Stanford, where it exceeds both parents in vigor. Possibly it could therefore find a suitable niche in the Coast Ranges of California, where it would be able to compete with other species.

III

MADIA CITRIGRACILIS

The *Eumadia* section of *Madia* contains as a subgroup a highly variable species complex of several closely related units native on the west coasts of both North and South America. The most widely known member of this complex is *Madia sativa* Mol., a species often found as a weed in various parts of the world and having 16 pairs of chromosomes (Johansen, 1933). *Madia gracilis* (Sm.) Keck, a more slender and smaller type, is one of the nearest relatives of *sativa*. Indeed, certain 16-chromosome forms of *gracilis* are difficult to separate from forms of *sativa* because of their close resemblance and similar behavior. These species intercross fairly readily and produce hybrids that are partially fertile. Their chromosomes are homologous, as is indicated by regular pairing, but the partial sterility of the hybrids shows that the chromosomes are not freely interchangeable.

Madia gracilis has been known for a century under the name *M. dissitiflora* (Nutt.) T. et G., but it was originally described by Sir James Edward Smith as a species of *Sclerocarpus*, a fact which was overlooked until recently (Keck, 1940). It is a very common plant on relatively dry soils from the Upper Sonoran up to the Hudsonian life zone, and is distributed from the Canadian border southward through the western United States to central Utah, Nevada, and northern Baja California, as is shown on the map, figure 44 (p. 41). It differs from the late-flowering, larger *M. sativa* in being principally a member of the spring flora and rarely a weed.

In the first period of these investigations a number of races of *gracilis* were cytologically examined and found to have 16 pairs of chromosomes. These races were widely distributed, but all were from relatively low elevations. Then a montane race, which was originally thought to belong to this species, was discovered to have 24 pairs of chromosomes. This race was grown from seed taken from a herbarium specimen collected by Frank W. Peirson on Burney Mountain, Shasta County, California, an area from which this species was not otherwise known. During a visit to this locality in 1938 additional material was obtained, which also had 24 pairs of chromosomes.

When this difference in chromosome number was first discovered, the 24-chromosome form was inspected closely for characters whereby it might be distinguished from the 16-chromosome form. Its rosettes and lower leaves were found to have a denser, softer, appressed pubescence, and the cauline leaves had a wider, somewhat more flaring base. Also, its central stem was overtopped considerably by the lateral branches, whereas in the 16-chromosome form the central stem equals or even overtops the laterals.

The discovery of a 24-chromosome *Madia* was of evolutionary interest, as this is the highest number found in any species of the Madiinae. The next highest is $n = 16$ of *Madia gracilis* and *sativa.* Other relatives in *Madia* have 8 pairs of chromosomes. Accordingly, these obviously related species are members of a polyploid series with a basic number of 8. The common form of *Madia gracilis* is therefore tetraploid, and the new form hexaploid.

It is very unlikely that the hexaploid form would arise from a tetraploid by autoploidy, for doubling of the chromosomes of the tetraploid would result in an octoploid with 32 pairs. Accordingly, this 24-chromosome form was suspected of having arisen from a cross between an 8- and a 16-chromosome species.

Comparison of the new species with its supposed parents. On account of their close morphological resemblance, *Madia gracilis* was thought to have been the 16-chromosome parent of the 24-chromosome form. On the basis of morphology, distribution, chromosome numbers, and known genetic behavior of *Madia* species, *M. citriodora* Greene was considered the only possible 8-chromosome ancestor in the present flora. Accordingly, the 24-chromosome material from Burney Mountain was given the provisional name *Madia citrigracilis* after its supposed parents.

Until the experimental investigations were made, *Madia citriodora* had never been thought to be a close relative of the *Madia sativa* complex. Rather, it had been thought closer to *M. elegans* D. Don, $n = 8$, principally because both species have sterile disk akenes. Asa Gray had even transferred *citriodora* from *Madia* to *Hemizonia* because of its broad ray akenes that were an exception to the key difference between these genera. Unlike *elegans* but like *gracilis,* however, it has inconspicuous, pale rays and is self-incompatible, and even from its morphology, *citriodora* is now universally accepted as a species of *Madia.*

Madia citriodora is a spring-flowering species like *gracilis.* Even though it has somewhat more showy ray florets and occupies similar habitats, yet it is much less frequently collected and must be regarded as comparatively rare. Its distribution, which extends from southern Washington to northern California in relatively dry environments east of the Cascade Mountains, is shown in figure 44 (p. 41).

An analysis of the more important morphological differences between *Madia citriodora*, *gracilis*, and *citrigracilis* is given in table 4. It is evident from this table that in most of its vegetative characters the new species resembles the diploid *citriodora*, but in the characters of the flowers and fruit it is very similar to its tetraploid relative, *gracilis*. The differences in habit among the three species are illustrated by the upper row of plants in figure 19. Considering their chromosome numbers, it is the morphological similarities rather than the differences that are striking.

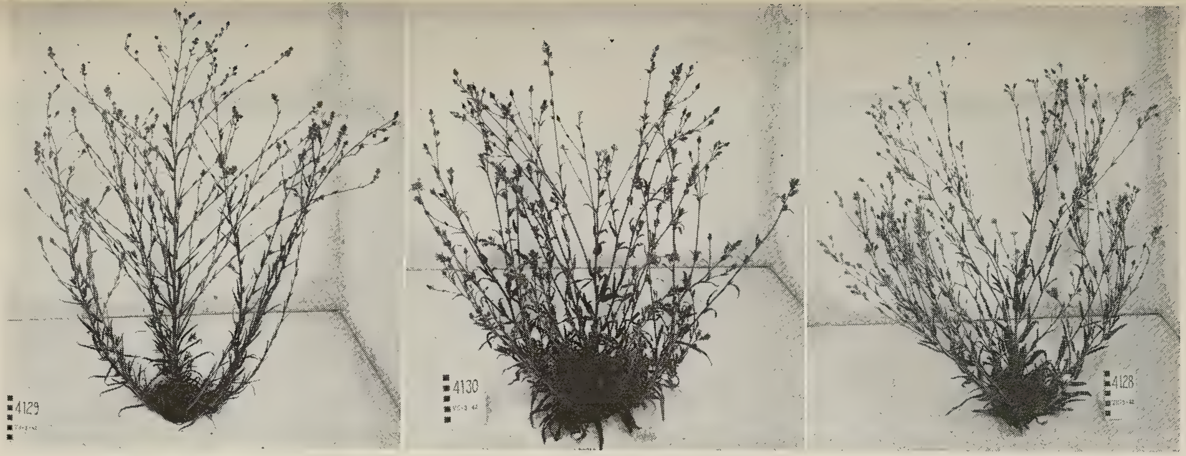
TABLE 4

Morphological differences between Madia citriodora, M. gracilis, and natural M. citrigracilis

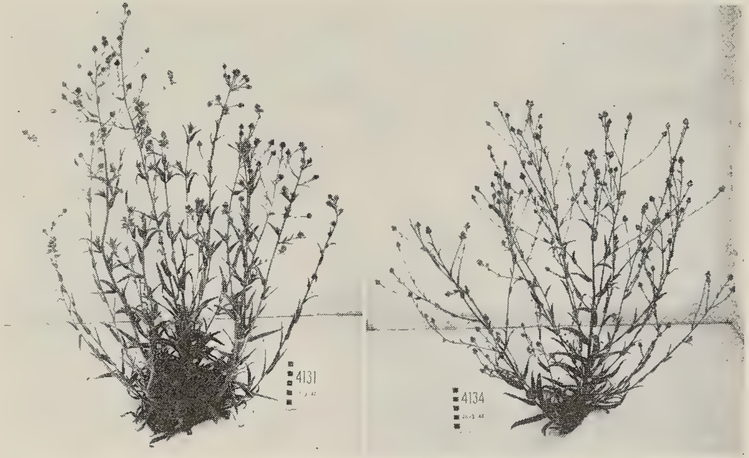
Character	*M. citriodora*	*M. citrigracilis*	*M. gracilis*
Side branches	surpassing main axis	surpassing main axis	shorter than or equaling main axis
Pubescence	densely villous, appressed	± densely villous, ± appressed	coarsely pilose, spreading
Herbage color	gray-green	gray-green	light green
Odor	lemon-scented	not lemon-scented	not lemon-scented
Stem leaves	broad at base	broad at base	narrow at base
Involucre	turbinate to urceolate	ovoid to globose	ovoid to globose
Ligule length	7–10 mm.	6–8 mm.	4–7 mm.
Receptacle	densely villous	glabrous or nearly so	glabrous or nearly so
Ray akenes	triangular in cross section	intermediate	laterally flattened
Disk akenes	sterile	fertile	fertile
Chromosome number	$n = 8$	$n = 24$	$n = 16$

SYNTHESIS OF MADIA CITRIGRACILIS

The discovery of the hexaploid *Madia*, and the deduction that it possibly arose through amphiploidy from a crossing between the diploid *Madia citriodora* and the tetraploid *gracilis*, prompted an attempt to synthesize it through hybridization (Clausen, Keck, and Hiesey, 1937). A race of *citriodora* from Parker Creek, Warner Mountains, Modoc County, and one of *gracilis* from the Middle Fork of Smith River, Del Norte County, from the two northern corners of California, were selected for crossing. Both were typical of their respective species, al-



gracilis, n=16 natural citrigracilis, n=24 citriodora, n=8



artificial citrigracilis, n=24

Fig. 19. *Above: Madia gracilis* from Middle Fork of Smith River, Del Norte County, California; *M. citrigracilis* from Burney Mountain, Shasta County; and *M. citriodora* from Parker Creek, Warner Mountains, Modoc County. *Below:* Plant 4131, F_3 amphiploid derivative of he *gracilis* and *citriodora* races shown above; and plant 4134, a spontaneous F_3 amphiploid of different parentage. (All plants in a uniform garden at Stanford.)

though the progenitors of the wild hexaploid could not have come from so widely separated localities.

Mechanical difficulties offer problems in emasculation of these regularly self-fertile species, because pollen is shed very early in the tiny, very glandular buds. Since some outcrossing does take place, however, open pollination in isolation plots was resorted to in the hope of obtaining hybrids. In 1937, in a plot well away from other species of *Madia*, one plant of *gracilis* was surrounded by a circle of seven plants of *citriodora*, and in another, one plant of *citriodora* was surrounded by seven plants of *gracilis*. In each plot the seeds of the central plant were harvested.

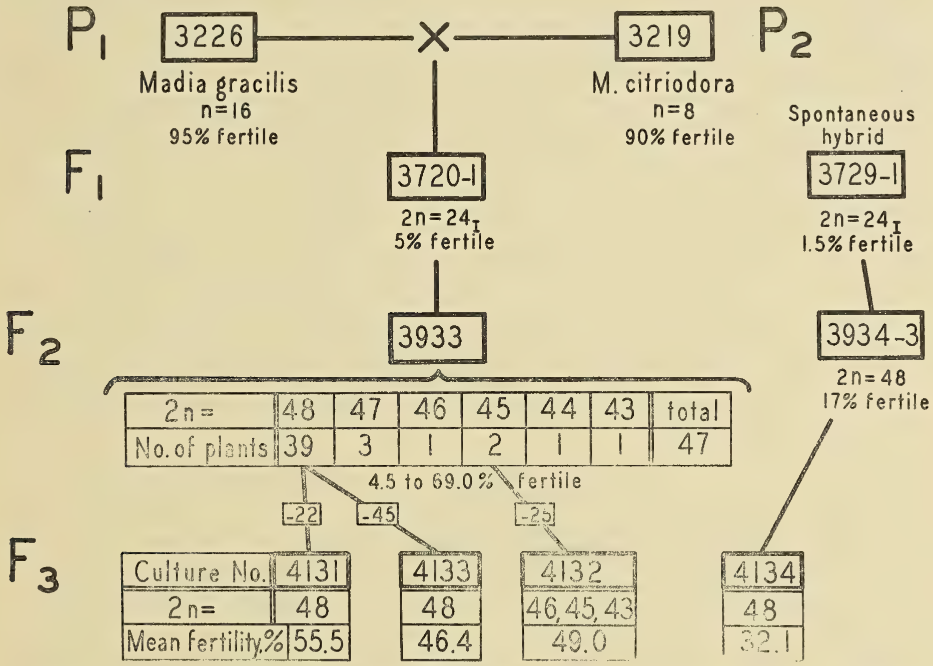


FIG. 20. Pedigrees of *Madia citrigracilis*, showing parentage, number of plants, chromosome numbers, and fertilities.

From the plant of *citriodora* approximately 400 akenes were obtained, from which, in 1940, only 34 plants were grown to maturity. All these proved to be selfed *citriodora*. About 1500 apparently good akenes were harvested on the *gracilis* parent in the reciprocal cross, and part of these were sown. They produced a culture of 399 plants, one of which, 3720-1 of figure 20, proved to be the desired F_1 hybrid. The others were selfed *gracilis*.

THE F_1 GENERATION. The hybrid was recognized among the selfed plants of *gracilis* by its wider leaf bases, its denser, more appressed pubescence, its longer side branches, and its faint *citriodora* odor. This plant lacked the alternate arrangement of the branches typical of both parents, for it was repeatedly subverticillate or subumbellate, a peculiarity often accompanied by a degeneration of the terminal bud of the central leader. This character was reproduced in later generations, but was not encountered in other hybrids between *citriodora* and members of the *sativa* complex. The final proof of the hybrid origin of this plant was found in its chromosome number, $n = 24$.

The F_1 plant was self-pollinated in an insect-proof greenhouse, and although it was quite highly sterile, a few akenes developed from both ray and disk florets. The disk akenes of *citriodora* are sterile, whereas those of *gracilis* are fertile. Therefore, the partial fertility of the disk akenes in the F_1 hybrid indicates that fertile disks are at least partially dominant over sterile disks, as they were in the *Madia nutans* × *Rammii* cross. Fertility of disk akenes has been employed as a specific, sectional, and even generic character in the Madiinae. It is consequently noteworthy that characters of such taxonomic importance are inherited in a manner similar to those used for distinguishing minor subspecific categories. Such evidence points to a fundamental similarity in the nature of the differences that distinguish both the large and the small taxonomic categories in plants.

THE F_2 POPULATION. Among some 1900 akenes harvested on the single first-generation hybrid, 94 appeared to be good, indicating a fertility of approximately 5 per cent. The following year these produced a second generation of 47 plants. This F_2 was quite uniform and faithfully reproduced the characters of the F_1, even to the peculiar subumbellate branching. The variation was of a minor nature and involved such characters as the citric odor of *citriodora*, which was present in varying degree in different plants and completely undetectable in others, and the slight dwarfing of a few individuals. On the whole, however, the culture consisted of plants as tall and vigorous as those of the parental species and of the natural form of *citrigracilis.*

This F_2 population was morphologically rather similar to garden plants of natural *citrigracilis.* The latter had paniculate instead of subumbellate branching above, no citric odor, and heads less strongly glandular. In more fundamental flower and fruit characters the two forms were essentially the same.

Among the 47 F_2 plants, 39 were fully amphiploid, with $2n = 48$ chromosomes, double the number of the F_1 hybrid. The remaining 8 plants varied in chromosome number between $2n = 43$ and 47, so they also were nearly amphiploid. This is shown in the pedigree, figure 20. Almost all the functional sex cells of the F_1 hybrid, therefore, must have contained all the chromosomes of both *gracilis* and *citriodora.*

The F_2 plants are listed in table 5, on page 32, and belong to three cultures, 3933 to 3935. The last two of these were derived from spontaneous hybrids to be mentioned later. All the plants of 3934 and 3935 and the plants 3933-1 to -19 were kept in a screened greenhouse to test their self-fertility, but the others were planted in the garden. It was found that the protected plants in the greenhouse were as fertile as those pollinated in the garden.

Forty-three of the F_2 plants of 3933 matured so that their fertility could be determined. As is shown in table 5, this varied between 4.5 and 69.0 per cent, averaging 41.6 per cent. This is a substantial increase from the 5 per cent fertility of the F_1 hybrid, and also much higher than the fertility of 5.5 per cent observed in the F_2 plants of *Madia nutrammii.* Even the eight plants with less than 48 pairs of chromosomes were from 4.5 to 62.0 per cent fertile, averaging 33.6 per cent. As in *gracilis* and natural *citrigracilis*, the ray and disk akenes were equally fertile.

SPONTANEOUS AMPHIPLOIDS BETWEEN MEMBERS OF THE M. SATIVA COMPLEX AND M. CITRIODORA. Three additional hybrids arose spontaneously in the garden as a result of insect pollination between *M. citriodora* and members of the *sativa* complex. They were detected by their morphological characters, their odor, and their chromosome number (24 somatic chromosomes instead of 32). The *Madia gracilis* parents of these hybrids were themselves sister intraspecific hybrids between the rare broad-akened form that has been described as *M. anomala* Greene and the common narrow-akened form, which are completely interfertile. These crossings took place in two different years, but the three hybrids were discovered in 1940 when many cultures of *Madia* were grown. The hybrids were as follows:

1. Plant 3729-1: *Madia gracilis* × *M. citriodora* F_1 (partial pedigree in fig. 20). The *citriodora* parent could have been either the Parker Creek strain, from Modoc County, or one from near Timbered Crater, in Siskiyou County. The F_1 hybrid was 1.5 per cent fertile; it resembled very closely the F_1 hybrid 3720-1 described above, and had similar cytological characteristics. From unprotected heads of this plant, pollinated in the garden, four F_2 offspring (3934) were obtained, three having $2n = 48$ chromosomes, and one $2n = 50$ (see table 5). The fertilities of these F_2 plants were much lower than those of the amphiploids of the previous hybrid. The plant with the highest fertility, 17 per cent, was

3934-3, with 48 regularly paired chromosomes. A vigorous F_3 generation, 4134 in the pedigree (fig. 20), was obtained from this plant, which was selfed in a screened greenhouse. Only part of the akenes were sown, but approximately 250 of them germinated. One of these plants is pictured in figure 19, in the lower row to the right. Unlike the previous amphiploid, the derivatives of this lineage resembled native *citrigracilis* from Burney Mountain in their branching. Two plants that were investigated were found to have $2n = 48$ chromosomes. The fertilities of twenty plants varied between 2 and 74 per cent, with an average of 32.1 per cent, a considerable improvement over the 17 per cent of the F_2 plant.

2. Plant 3737-1: *Madia (gracilis × sativa) × citriodora*, Parker Creek, F_1. The maternal parent was an F_1 hybrid between a plant of the *gracilis* parentage mentioned above ($n = 16$) and the closely related *sativa* ($n = 16$). The chromosomes of *gracilis* and *sativa* pair regularly, but some genetic incompatibility between these species is indicated by the fact that their hybrids are only 3 to 30 per cent fertile, depending upon the races crossed. In spite of the fact that the maternal genome was a mixture from two forms of *gracilis* and one of *sativa*, this hybrid behaved like 3720-1, which contained a maternal genome of only one species. The influence of the genes of *sativa* was found in the slightly more robust and more densely glandular stems as compared with the two F_1 hybrids described above. This hybrid was completely sterile, for no good akenes were found among approximately 1500 empty and undeveloped ones.

3. Plant 3737-2: Same origin as 3737-1, described above. Although this hybrid appeared to be identical morphologically with its sister plant, it differed in being 3.1 per cent fertile, for among some 350 akenes, 11 apparently good ones were found, from which seven F_2 plants were grown, all with $2n = 48$ chromosomes. This F_2 population is culture 3935 of table 5 (p. 32). All its plants were straight amphiploid, and their fertilities varied between 12 and 49 per cent, but no F_3 was grown from it. That this amphiploid contained some *sativa* genes was evident from the inflorescences, which differed from the others in being less branched and in having larger, more congested heads. The stems were also coarser and their upper leaves had a wider base than in the preceding hybrids. This amphiploid lacked the odor of *citriodora* (although the F_1 parent had it), and, like the offspring of 3729-1, it closely approached native *Madia citrigracilis* in habit.

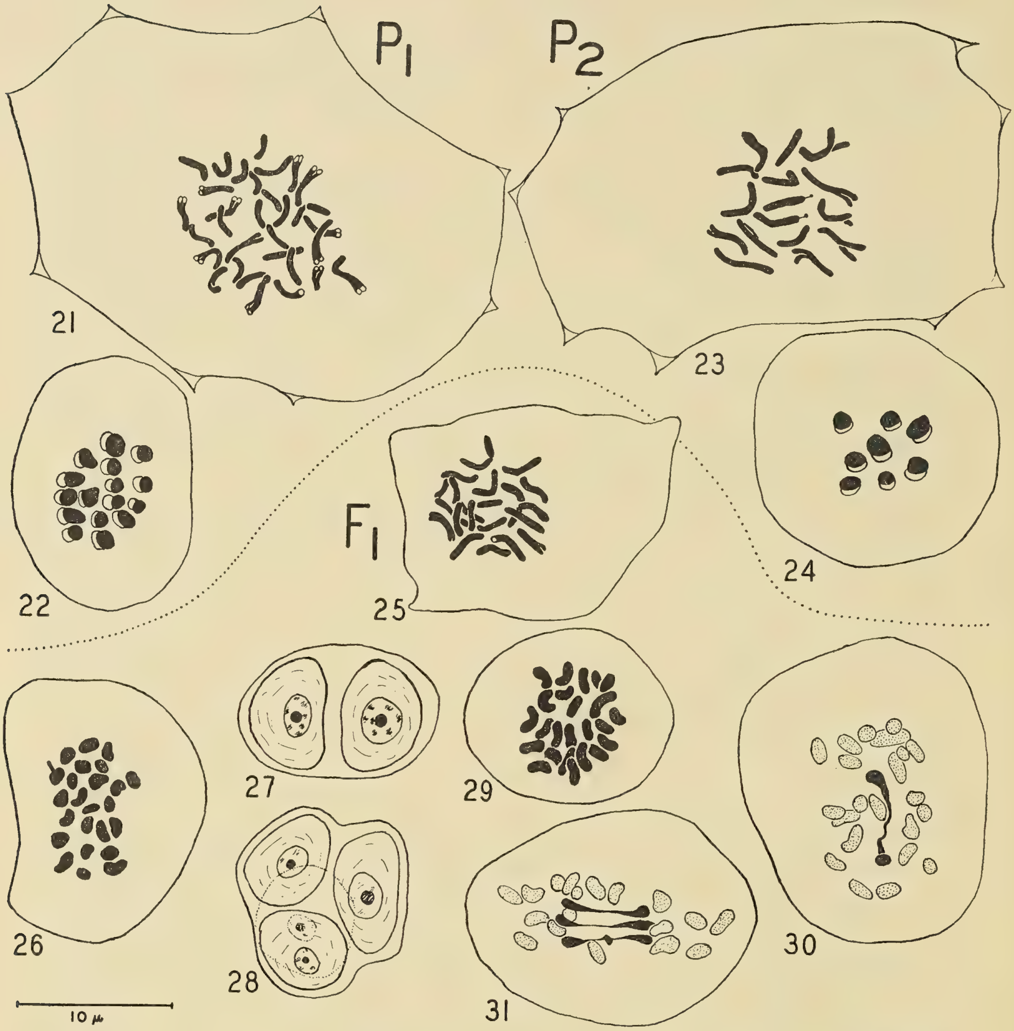
Considerable other experimental evidence points to the close cytogenetic relationship between *sativa*, *gracilis*, and the *anomala* form of *gracilis*. Therefore it is not so surprising that the different combinations of their chromosomes in a hybrid did not interfere with the production of an amphiploid when a genome of *citriodora* was introduced. It appears that the combinations of forms of *gracilis* or *sativa* with *citriodora* produce only amphiploids, because three F_1 plants of considerably different parentage have produced 58 F_2 offspring, of which 49 are straight amphiploids and the remainder deviate but slightly.

CYTOLOGY OF MADIA CITRIGRACILIS AND THE PARENT SPECIES. Pairing of the chromosomes is perfectly regular in both parents of the artificial amphiploid. The 16 pairs of *gracilis* are somewhat smaller, on the average, than the 8 pairs of *citriodora*, but the differences were scarcely large enough to be recognized. Somatic and meiotic chromosomes of the *gracilis* parent are shown in figures 21 and 22, and the corresponding plates of the *citriodora* parent in figures 23 and 24. There is apparently no significant difference in the size of the cells of the two species, for *citriodora* has remarkably large cells for a diploid.

Suitable material for cytological study of the first-generation hybrids between *gracilis* and *citriodora* was fairly scarce, because buds are small when meiosis takes place, and the best material was too old by the time the hybrids were mature enough to be recognized. The study of the pairing of the chromosomes in the F_1 hybrids has therefore been made on all the available bud material, including that from the artificially produced F_1 plant (3720-1), and from two of the spontaneous hybrids (3737-1 and -2). The meiotic behavior of the chromosomes was essentially the same in all three.

Very little pairing of chromosomes was observed in the F_1 hybrids. Occasionally one or two (very rarely three or four) very loose pairs could be observed in first metaphase. Very commonly, however, all chromosomes were single, although they aligned in a fairly regular equatorial plate. Obviously, the chromosomes of *citriodora* are not homologous with any of those of *gracilis*.

In the comparatively fertile artificial hybrid, 3720-1, no pairing was observed, but suitable material of this plant was very scarce. A somatic cell in the floral region had 24 chromosomes (fig. 25), which indicates that this plant was a hybrid and that it had not doubled its chromosomes somatically. Meiosis was found in only one anther lobe, which contained 9 to 10 cells. These were in metaphase, each with one chromosomal plate, and 24 chromosomes could be counted (fig. 26). Side views gave the impression that 24 pairs were present, but this interpretation is excluded because the somatic number was only 24. These cells were there-



Figs. 21–31. Somatic and meiotic chromosomes of *Madia gracilis* (P_1), *M. citriodora* (P_2), and F_1 hybrids. Univalent chromosomes are stippled. Further explanation in text. × 2000.

fore interpreted as being in a stage corresponding to second metaphase of restitution nuclei resulting from complete nondisjunction during first metaphase. No cells in typical second metaphase were found in this lobe to support such an interpretation, but both dyads and tetrads were observed in this plant (figs. 27, 28). In a study of 46 microspore groups in anther lobes, 20 tetrads, 23 dyads, and 3 triads were found. Both random distribution and nondisjunction therefore occurred in almost equal proportions, and the high percentage of diploid sex cells explains the comparatively high fertility of this hybrid. The pollen was extremely variable in size, ranging from very large to minute grains.

No stages early enough to permit observation of meiosis were found in the spontaneous hybrid 3729-1, but pollen very variable in size was observed, and this plant's amphiploid offspring in the cultures 3934 and 4134 are sufficient evidence that its meiosis was comparable to that of 3720-1.

Good first metaphases but no second metaphases were observed in both of the spontaneous hybrids of 3737, which carried some *sativa* genes. Pairs were frequently observed in them, and even four loose pairs were seen in one cell of each. Figures 29 to 31 illustrate first metaphases of the fertile hybrid 3737-2, which produced the culture 3935 with seven amphiploids. In two of these cells the singles are scattered over the spindle surrounding the very loose pairs. Dyads and tetrads were found intermixed in the anther lobes, and great differences in size were seen in the mature pollen, ranging from dwarf to very large and well developed grains. It is therefore evident that the patterns of pollen development were quite similar in all four F_1 hybrids despite their differences in origin. Judging from the offspring obtained, only the diploid or near-diploid sex cells survived.

CYTOLOGY OF THE SECOND-GENERATION OFFSPRING. The chromosomes of both root tips and pollen mother cells were studied in all the F_2 plants so far as material was available. The resultant counts are listed in table 5 for the F_2 individuals of the synthesized hybrid, 3720-1, as well as for the eleven F_2's originating from the two partially fertile spontaneous hybrids. In addition to the somatic and meiotic numbers, the mode of pairing during meiosis and the fertility of each plant are listed. The fertility figures were determined on a sample of 200 akenes from twenty or more ripe heads.

The second generation of *Madia gracilis* × *citriodora* was cytologically much more regular than that of *nutans* × *Rammii*. Out of the total

TABLE 5

NUMBER AND PAIRING OF CHROMOSOMES AND FERTILITY IN F_2 PLANTS OF MADIA GRACILIS × M. CITRIODORA

PLANT NUMBER	2n	PAIRING IN FIRST METAPHASE		FERTILITY OF AKENES (PER CENT)
		Usual	Deviation	
OFFSPRING OF 3720-1				
3933-1	48	24_{II}	$23_{\text{II}} + 2_{\text{I}}$	42.5
-2	48	not seen	....	44.0
-3	48	24_{II} + fragment	$23_{\text{II}} + 2_{\text{I}}$ + fragment	died
-4	48	....	....	died
-5	47	$23_{\text{II}} + 1_{\text{I}}$	$22_{\text{II}} + 3_{\text{I}}$	27.5
-6	48	24_{II}, regular	....	56.0
-7	48	24_{II}, regular	....	13.5
-8	48	24_{II}, regular	....	51.5
-9	48	24_{II}, regular	....	43.5
-10	43	$20_{\text{II}} + 3_{\text{I}}$	$19_{\text{II}} + 5_{\text{I}}$	36.0
-11	48	24_{II}, regular	....	52.5
-12	48	not seen	....	54.0
-13	48	24_{II}	$23_{\text{II}} + 2_{\text{I}}$	47.5
-14	48	not seen	....	died
-15	48	24_{II}, regular	....	45.5
-16	48	24_{II}, regular	....	52.0
-17	48	24_{II}	$23_{\text{II}} + 2_{\text{I}}$	44.0
-18	48	24_{II}	$23_{\text{II}} + 2_{\text{I}}$	37.5
-19	48	24_{II}, regular	....	58.0
-20	48	not seen	....	38.0
-22	48	24_{II}	$23_{\text{II}} + 2_{\text{I}}$	69.0
-23	46	23_{II}	$22_{\text{II}} + 2_{\text{I}}$	52.5
-24	44	$21_{\text{II}} + 2_{\text{I}}$	....	35.0
-25	45	$22_{\text{II}} + 1_{\text{I}}$	$21_{\text{II}} + 3_{\text{I}}$	62.5
-26	45	$21_{\text{II}} + 3_{\text{I}}$	$20_{\text{II}} + 5_{\text{I}}$	10.0
-27	48	24_{II}	$23_{\text{II}} + 2_{\text{I}}$	39.5
-29	48	24_{II}, regular	....	47.0
-30	48	24_{II}	$23_{\text{II}} + 2_{\text{I}}$	26.0
-31	48	24_{II}	$23_{\text{II}} + 2_{\text{I}}$, $22_{\text{II}} + 4_{\text{I}}$	40.0
-32	48	24_{II}, regular	....	60.0
-33	47	$23_{\text{II}} + 1_{\text{I}}$	$22_{\text{II}} + 3_{\text{I}}$	47.0
-34	48	24_{II}, regular	....	26.0
-35	48	24_{II}, regular	....	32.5
-36	48	24_{II}, regular?	....	30.0
-37	48	24_{II}, regular	....	46.0
-38	48	24_{II}, regular	....	33.5
-39	48	24_{II}	....	32.5
-40	48	not seen	....	died

(*Continued on following page*)

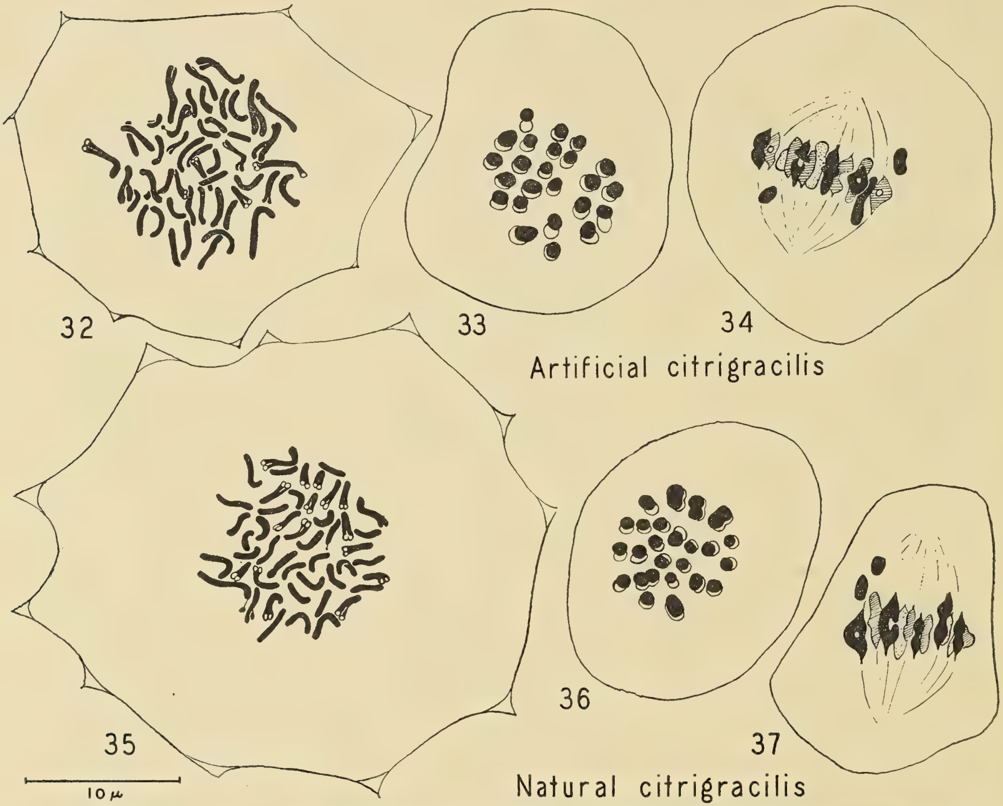
TABLE 5—*Continued*

PLANT NUMBER	$2n$	PAIRING IN FIRST METAPHASE		FERTILITY OF AKENES (PER CENT)
		Usual	Deviation	
3933-41	47	$22_{II} + 3_{I}$	....	4.5
-42	48	24_{II}	$23_{II} + 2_{I}$	41.0
-43	48	24_{II}	$23_{II} + 2_{I}$	53.5
-44	48	24_{II}	$23_{II} + 2_{I}$	42.5
-45	48	24_{II}	$23_{II} + 2_{I}$	58.0
-46	48	24_{II}	$23_{II} + 2_{I}$	26.5
-47	48	$23_{II} + 2_{I}$	$22_{II} + 4_{I}$	21.5
-48	48	24_{II}	$23_{II} + 2_{I}$	26.5
-49	48	24_{II}	$23_{II} + 2_{I}$	18.0
OFFSPRING OF 3729-1				
3934-1	48	$23_{II} + 2_{I}$	24_{II}	4.0
-2	48	24_{II}, regular	....	6.0
-3	48	24_{II}, regular	....	17.0
-4	50	$24_{II} + 2_{I}$	$23_{II} + 4_{I}$	10.0
OFFSPRING OF 3737-2				
3935-1	48	24_{II}, regular	....	49.5
-2	48	24_{II}, regular	....	26.0
-3	48	24_{II}, regular	....	43.5
-4	48	24_{II}, regular	....	33.5
-5	48	not seen	....	31.5
-6	48	24_{II}	$23_{II} + 2_{I}$	24.0
-7	48	24_{II}	$23_{II} + 2_{I}$	12.0

of 58 F_2 individuals listed in table 5, 49 had $2n = 48$ chromosomes. Pairing was generally regular in first metaphase except for the occasional occurrence of cells with 23 pairs and 2 singles, indicating a loose attraction between the members of one pair. This tendency was observed to be fairly frequent in 20 of the 49 plants. Figures 32 to 34 show somatic and meiotic chromosomes of a plant in this class.

The chromosomes of the natural hexaploid from Burney Mountain also occasionally show two univalents during first metaphase. This natural species, however, shows this type of irregularity much less frequently than does the average F_2 amphiploid of artificial origin. Somatic and meiotic plates, including one with 23 pairs plus 2 univalents, are shown in figures 35 to 37 in order to facilitate comparisons with those of the artificial hexaploid just above them.

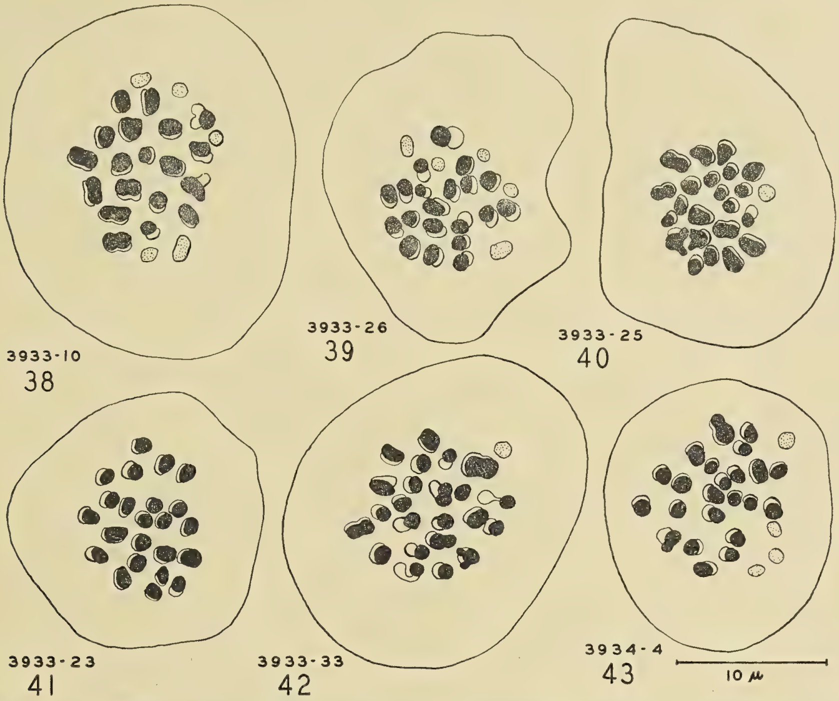
Chromosome plates are shown in figures 38 to 43 of aneuploid F_2 plants with 43, 45, 46, 47, and 50 chromosomes. The presence of these plants in the F_2 populations indicates that some sex cells with a slight deviation in chromosome number from the straight hexaploid were viable. Most of these showed no striking decline in either vigor or fertility as compared with some 48-chromosome plants. The individual 3933-23, which has 23 pairs of chromosomes instead of 24 (fig. 41), even suggests a way in which aneuploid numbers may arise from members of a polyploid series, for this plant was both reasonably fertile and vigorous. Although *citrigracilis* belongs to a series of species with strictly polyploid chromosome numbers, the genic balance of this group is more flexible on the hexaploid than on the lower levels, permitting survival and



FIGS. 32–37. Comparison of somatic and meiotic chromosome plates between artificial *Madia citrigracilis* (plant 3933-22) and the natural form from Burney Mountain. × 2000.

propagation of plants that deviate slightly from the strictly hexaploid number.

THE F_3 POPULATIONS. Akenes were harvested from each plant of the F_2, and four F_3 populations were grown the following year from selected plants. Three were derived from straight amphiploids with $2n = 48$, and one from a plant with $2n = 45$ chromosomes (fig. 20).



FIGS. 38–43. Polar views of first metaphase in meiotic divisions of six aneuploid *Madia citrigracilis* F_2 plants having, respectively, $2n = 43, 45, 45, 46, 47,$ and 50 chromosomes (cf. table 5). Univalents are stippled. × 2000.

From the three 48-chromosome plants approximately 650 seedlings were obtained, although only some of the akenes were sown. Part of these were transferred to the garden, where 201 plants matured. These were generally vigorous and even more uniform in appearance than the F_2. Table 6 indicates the vigor and uniformity of the combined 48-chromosome F_3 cultures and compares them with the parental species and natural *citrigracilis*. The height of the plants, when grown in a uniform garden, is found to be a satisfactory indication of vigor. Although the akenes for these cultures had been harvested from unprotected plants in the garden, no cross pollination had taken place, for the peculiarities of the strains had been faithfully reproduced. Two representative F_3 plants are shown in figure 19 in the lower row.

TABLE 6

FREQUENCY DISTRIBUTION OF HEIGHTS OF ARTIFICIAL MADIA CITRIGRACILIS, ITS PARENTS, AND NATURAL CITRIGRACILIS IN A UNIFORM GARDEN

Species	Class intervals (cm.)									Total plants	Mean height (cm.)
	25	35	45	55	65	75	85	95	105		
Madia citriodora, Parker Creek, $n = 8$	..	1	15	12	46	21	2	..		97	68.14
Madia gracilis, Smith River, $n = 16$	..	1	6	35	59	95	35	2		233	75.19
Artificial M. citrigracilis, F_3, $n = 24$	..	7	35	62	68	28	1	..		201	63.88
Natural M. citrigracilis, Burney Mountain, $n = 24$	3	19	34	62	26	..	..	..		144	56.18

A sample of five F_3 individuals from the three 48-chromosome parents was investigated cytologically. Each had $2n = 48$ and, like the F_2 parents, showed only slight irregularities in pairing.

The fertility of the 48-chromosome F_3 was not yet as high as in natural *citrigracilis*. A sample of twenty plants from each population was tested and found to vary between 2 and 84 per cent fertile, with means of 55.5, 46.4, and 32.1 per cent, as is indicated in figure 20. This is an advance over the F_2, which indicates that further improvement would be likely in subsequent generations, and that through natural selection artificially synthesized *citrigracilis* could doubtless become more uniformly successful. The fertilities already established are better than those found in some other species of *Madia*, and high enough to enable a single plant to yield about a thousand offspring.

In contrast with the F_3 progeny of the 48-chromosome parents, those of the 45-chromosome plant were distinctly weaker, slower in development, and more variable. The fertility of the parent had been determined as 62.5 per cent, but the germination was poor; out of 77 seedlings obtained, 34 died early, and among the 43 survivors only 23 flowered. The fertilities of these, as determined by the appearance of the akenes, ranged between 7 and 70 per cent, with a mean of 49 per cent.

Six F_3 offspring of the 45-chromosome plant were studied cytologically. Four had $2n = 46$, one $2n = 45$, and one $2n = 43$ chromosomes.

The preponderance of 46-chromosome plants in this small sample may indicate a tendency for the chromosome numbers to become established at this figure, but this strain appears unable to compete successfully with the 48-chromosome strains.

Comparison of artificial and natural Madia citrigracilis. The cytologically balanced derivatives of the artificial hexaploid appear to constitute a stable and successful race. In the garden they are on the average slightly smaller than their parental strains, but they are larger than the natural hexaploid from Burney Mountain (table 6) and than many races of *gracilis*. The artificial race is so similar morphologically and cytologically to natural *citrigracilis* as to bring very strong support to the theory that the natural hexaploid arose through amphiploidy from an original hybrid between *gracilis* and *citriodora*. The various lines of evidence in support of this theory may be summarized as follows: (1) both *citriodora* ($n = 8$) and *gracilis* ($n = 16$) occur in the same general region as the hexaploid ($n = 24$); (2) the artificially produced amphiploid matches natural *citrigracilis* in morphology, vigor, and cytological characteristics; (3) amphiploids between *citriodora* and *gracilis* have arisen spontaneously in garden cultures on three separate occasions, under conditions of pollination such as may exist in the wild; and (4) the existence of a cytological mechanism for doubling the chromosomes in the F_1 hybrid between these species is now clearly established.

The final link in the chain of evidence that would prove the conspecific nature of the hexaploids has not been attained. This involves the production of hybrids between the two hexaploids and a test of the fertility of these hybrids. Unfortunately, these forms are not suitable for crossing with emasculation, and their extreme morphological and cytological similarity essentially precludes the possibility of distinguishing between their hybrids and selfed offspring. The strong evidence makes it reasonably certain, however, that natural *citrigracilis* is an amphiploid of the origin suggested.

MADIA GRACILIS AS A POLYPLOID COMPLEX

The repeated production of *M. citrigracilis* from different races of the parental species in the garden suggested that it should be frequently found in the wild and not merely localized as at Burney Mountain. Furthermore, the morphological similarity between natural *citrigracilis* and *gracilis* from northern California needed to be considered before the hexaploid could be proposed as a taxonomic species. These facts led to an investigation of the chromosome numbers of the races of *Madia gracilis* that were in culture.

Counts were made of 58 populations from various localities, ranging from central Washington to southern California, and from near sea level up to 2380 meters elevation. This cytological survey showed that what had been passing for *M. gracilis* (*M. dissitiflora*) is in fact a polyploid complex, for among the 58 cultures studied, 2 were diploid ($n = 8$), 45 were tetraploid ($n = 16$), and 11 were hexaploid ($n = 24$). In table 7

TABLE 7

CHROMOSOME COUNTS IN THE MADIA GRACILIS COMPLEX

Locality	Elevation (meters)
Madia subspicata, $n = 8$	
California:	
SW. of Richardson's Springs, Butte Co	150
Wildcat Canyon, Stanislaus Co	50
Tetraploid Madia gracilis, $n = 16$	
British Columbia:	
Thames Creek, Vancouver Island	25
Idaho:	
Thatuna Hills, Latah Co	610
Washington:	
Bingen, Klickitat Co	30
Oregon:	
Hood River, Hood River Co	30
Grants Pass, Josephine Co	290
Mount Ashland, Jackson Co	1675
California:	
Davis Creek, Modoc Co	1525
Castella, Shasta Co	600
Lake Almanor, Plumas Co	1340
Quincy, Plumas Co	1070
N. of Nevada City, Nevada Co	850
Grass Valley, Nevada Co	730
Baxter's Camp, Placer Co	1220
American River Canyon, Eldorado Co	1160
Dry Creek, N. of Eldorado, Eldorado Co	425
SW. of Plymouth, Amador Co	305
N. of Sutter Creek, Amador Co	350
*N. of Jackson, Amador Co	425
Vallecita, Calaveras Co	550
Angels Camp, Calaveras Co	455
Priest, Tuolumne Co	800
E. of Groveland, Tuolumne Co	890

(*Continued on following page*)

TABLE 7—*Continued*

Locality	Elevation (meters)
Smith River, Del Norte Co	100
W. of Weaverville, Trinity Co	425
W. of Willow Creek, Humboldt Co	240
Buck Mountain, Humboldt Co	760
S. of Weott, Humboldt Co	60
N. of Willits, Mendocino Co	430
Clear Lake Oaks, Lake Co	365
*Napa Range, Napa Co	335
E. of Muir Beach, Marin Co	90
*Mill Valley, Marin Co	30
South San Francisco, San Mateo Co	3
W. of Belmont, San Mateo Co	120
*La Honda, San Mateo Co	125
*Grant's Ranch, Mount Hamilton, Santa Clara Co	450
Hall's Valley, Mount Hamilton, Santa Clara Co	455
Llagas Creek, Santa Clara Co	165
N. of Ben Lomond, Santa Cruz Co	105
Tularcitos Creek, Monterey Co	230
San Luis Obispo, San Luis Obispo Co	60
Nojoqui Pass, Santa Barbara Co	275
San Marcos Pass, Santa Barbara Co	850
Mandeville Canyon, Los Angeles Co	535
San Dimas Canyon, Los Angeles Co	535
Hexaploid Madia gracilis, $n = 24$	
Washington:	
N. of Ellensburg, Kittitas Co	500
California:	
Modoc Lava Beds, Modoc Co	1430
Mount Shasta, Shasta Co	1525
Near Lake Tahoe, Eldorado Co	1920
Cottonwood Meadow, Yosemite National Park	1830
Dark Hole, Yosemite National Park	2375
Mariposa Grove, Yosemite National Park	1890
South Fork Mountain, Humboldt Co	1680
Chews Ridge, Monterey Co	1480
Chews Ridge, Monterey Co	1540
Jolon Grade, Monterey Co	455
Madia citrigracilis, $n = 24$	
California:	
Burney Spring, Shasta Co	1465
Burney Spring, Shasta Co	1430

*Chromosome counts made by Donald A. Johansen (1933).

the places of origin of these populations and of *citrigracilis* are listed, and in figure 44 their general location is indicated.

The two diploid populations had previously been recognized in these investigations as members of a morphologically distinct unit, called by us *subspicata*, but not thought to be of specific rank. These plants are very low, with very short lateral branches, if any, and the heads are arranged in a spikelike raceme; the herbage is yellow-green, and the plants bloom exceptionally early in the spring. This form is sporadic and rare, thus far having been collected only at half a dozen localities in the blue oak belt in the lowest foothills of the Sierra Nevada. The two cultures whose chromosomes were counted originated from localities 150 miles apart, but nevertheless they were morphologically indistinguishable. The sporadic occurrence of the diploid suggests that its present-day colonies may be relicts of a once continuous distribution. When cultivated in a uniform garden these forms are fairly distinct in appearance as compared with other members of the *gracilis* complex.

Very few colonies of tetraploid *Madia gracilis* in the Pacific states occur above 1000 meters, and their upper limit appears to be around 1400 meters. Morphologically these plants are extremely variable, probably in part because of occasional gene exchange with the equally tetraploid *Madia sativa* of the Coast Ranges. Although tetraploid *gracilis* is morphologically fairly similar to the diploid *subspicata*, the cytological evidence suggests that it is not an autoploid, for the 16 *gracilis* chromosomes in the *gracilis* × *citriodora* hybrid do not pair inter se. In spite of the morphological resemblance between *gracilis* and *subspicata*, their chromosomal difference indicates that they are genetically distinct species.

The hexaploids occur almost exclusively at higher elevations (mainly above 1400 meters). They have been found to occur from central Washington to the central Sierra Nevada, and also in the Santa Lucia Mountains of the Coast Range. The occurrence of *gracilis* at these elevations in mountain masses as far east as central Idaho and Utah and as far south as the Tehachapi region of California suggests that hexaploid forms may extend to these areas. Two collections of the hexaploid from low elevations are listed in table 7. One of these is from Jolon Grade, where it may have come down from near-by localities in the higher Santa Lucia Mountains. The other is from a wash north of Ellensburg, Washington, but the species is not on record from the mountain masses surrounding that locality.

There are no good morphological characters by which the hexaploid forms of *Madia gracilis* can be distinguished from the tetraploids. Like other annual plants occurring at elevations above 1500 meters, they are, on the average, more slender and shorter than the tetraploids from lower elevations. Also, they tend to be slower in development and much more

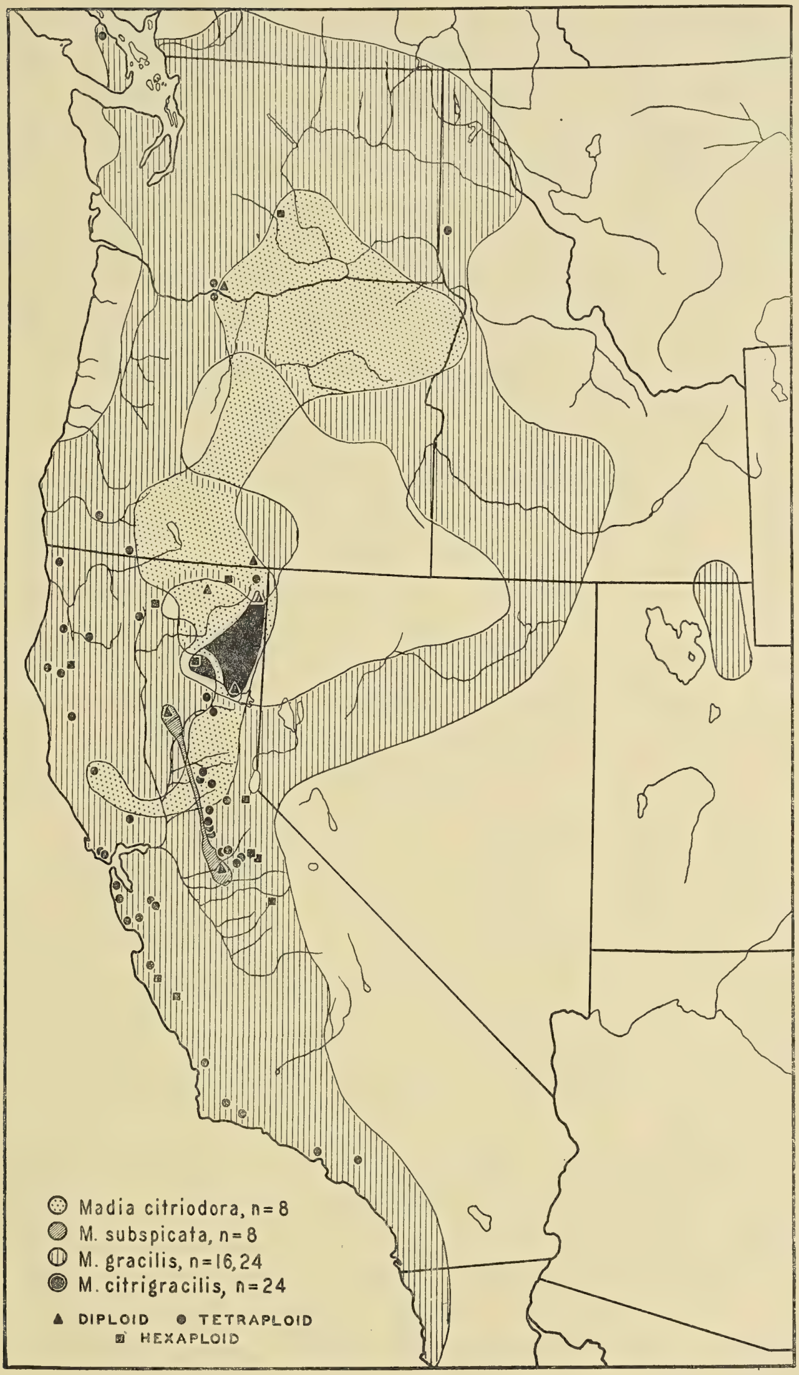


FIG. 44. Distribution of members of the *Madia gracilis* complex. The localities from which chromosome counts have been made are indicated.

difficult to germinate at Stanford. The hexaploids from the Santa Lucia Mountains, however, do not differ from the tetraploids from the same region either in size or in seasonal reactions. Although genetically determined, the characteristics of this southern material are evidently correlated with the climatic zone in which they are native rather than with chromosome number.

Hexaploid *Madia gracilis* without any doubt constitutes a genetic species distinct from tetraploid *gracilis*, but the two cannot be told apart except by chromosome number and distribution. One of the most extreme cases of a similar kind is in *Viola Kitaibeliana* Roem. et Schult., in which there are no morphological means by which forms with 7, 8, 18, and 24 pairs of chromosomes can be distinguished (J. Clausen, 1931*b*).

Difficulty is also encountered in parts of northern California in distinguishing between *Madia citrigracilis* and hexaploid forms of *M. gracilis*. It is apparent that some gene exchange has taken place between these units. A careful examination of herbarium material shows that *citrigracilis* from Burney Mountain in Shasta County is duplicated by plants of several collections from Lassen County, and probably the same form is found in eastern Modoc County. The collections of hexaploid and supposedly hexaploid forms westward toward Mount Shasta are more like *gracilis*, although they approach *citrigracilis* in certain characteristics. The probable *citrigracilis* influence is seen especially in paniculate inflorescences, larger and wider akenes, and sometimes also in the pubescence. Some populations, such as the one from Mount Shasta, are quite variable and contain, besides typical *gracilis*, individuals that approach *citrigracilis*. Still farther westward, through Siskiyou and Trinity counties to eastern Humboldt County, the material from these elevations is progressively more typical of *gracilis*. The possibility must therefore be considered that *citrigracilis* may have arisen several times in the wild, but that it has lost its identity through crossings with hexaploid *gracilis* except in Lassen and Shasta counties, where it appears to be isolated and unadulterated.

Madia citrigracilis and hexaploid *gracilis* are apparently of different origin, although both probably arose through amphiploidy. The pairing of the chromosomes is perfectly normal in hexaploid *gracilis*, with no evidence of multivalent association. Also, an autoploid *gracilis* would be expected to have 32 instead of 24 pairs of chromosomes. Possibly hexaploid *gracilis* arose through crossing of the now nearly extinct diploid *subspicata* with tetraploid *gracilis*, followed by chromosome doubling, although the possibility also exists that its diploid ancestor was some now extinct species morphologically rather similar to *subspicata*.

The situation found in this complex may be illustrated by a diagram (fig. 45). Five biological units exist at three levels of polyploidy: two diploids, one tetraploid, and two hexaploids. Unbroken lines indicate the relationship between *citrigracilis*, *gracilis*, and *citriodora* as established by experiment, and the broken lines indicate other possible relationships arrived at by circumstantial evidence.

The classification of the five biological units in the *gracilis* complex should be considered from three distinct viewpoints. From the phylogenetic point of view all five are equally important, for each has undoubtedly had a different origin and history, but we may expect never to be able to discover more than a fraction of the history of such a group. From the genetic viewpoint, the two diploids and the tetraploid are all distinct species, but the two hexaploids might have to be considered as one species, since there is some circumstantial evidence of interbreeding. From the morphological point of view, only *citriodora* is very clearly set off from the four others, although *subspicata* and *citrigracilis* are distinguishable from the two *gracilis* forms.

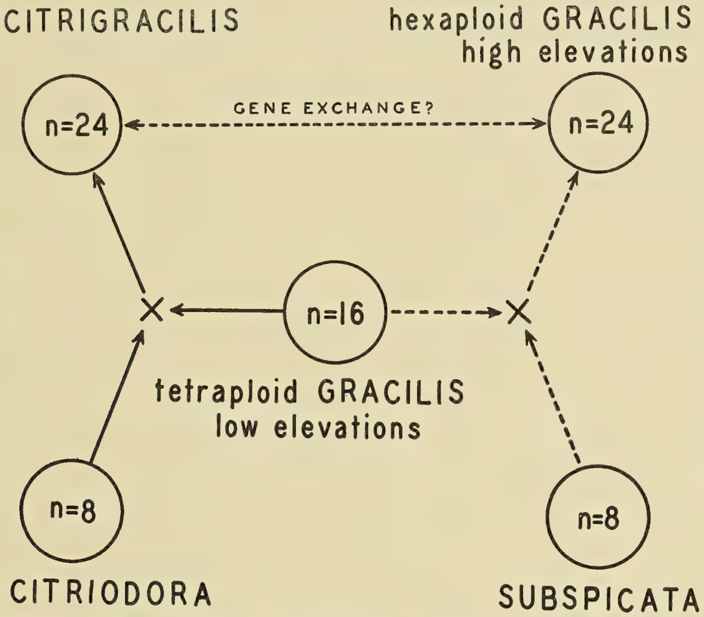


Fig. 45. *Madia gracilis* and allies. Relationships arrived at by experiment indicated by solid lines; those by circumstantial evidence, by broken lines.

In a case like this, where the three points of view do not lead to the same conclusion, the classification should preferably be practical. At present, it is impossible to distinguish tetraploid from hexaploid *gracilis* by morphological means. A practical solution for this problem in classification is therefore to include both of these in one taxonomic species, *Madia gracilis*, but to recognize two diploid species, *citriodora* and *subspicata*, and also to maintain as a species the hexaploid *citrigracilis*. In this fourth species of the complex there has been included only that material from Modoc, Lassen, and Shasta counties that closely matches the type collection and therefore also the synthesized *citrigracilis*. The hexaploids from Siskiyou County, that have some characteristics of *gracilis* and some of *citrigracilis*, are considered members of *gracilis*.

Madia citriodora, *subspicata*, tetraploid and hexaploid *gracilis*, and *citrigracilis* clearly constitute an example of a sexual polyploid complex (Babcock and Stebbins, 1938), and of the biological problems that may be found in such a group. Still other species with problems almost equally intricate belong to this same cenospecies, but their exposition is withheld for a later publication on this genus.

SYSTEMATIC NOTES

Madia citrigracilis Keck sp. nov.

Annua, 25–50 cm. alta; caule deorsum hispido-hirsuto superne villoso et viscido-puberulo cum prominenti stipitatis glandulis ornato, subsimplici vel (praecipue superne) stricte paniculato-ramoso; foliis lineari-oblongis, inferioribus 4–8 cm. longis 4–6 cm. latis, basi saepe subamplexicaulibus; capitulis stricte racemosis vel ad apices ramorum solitariis; involucro obovato 6–8 mm. alto, squamis lineari-lanceolatis aliquanto hirsutis et dense stipitato-glandulosis, marginibus villoso-ciliatis, apice acuminato; corollis radii 5–14, ligulis inconspicuis 5–8 mm. longis, tubo moderate pubescente et viscido-puberulo 1.5 mm. longo gracile; corollis disci 8–18 (–30), 2.5–3 mm. longis, tubo et lobis brevi-pubescentibus viscidisque; antheris nigris; acheniis radii ca. 4 mm. longis minute striatis muriculatisque nigris vel brunneo-maculosis late oblanceolatis (late lanceolatis in sectione) paullo arcuatis (nervo intus vix incurvo), areola terminale subsessile; acheniis disci fertilibus ad achenia radia similibus. $n = 24$.

Type, from Burney Spring, south side of Burney Mountain, Shasta County, California, at 1465 m. elevation, under *Pinus ponderosa* in volcanic ash, where not very plentiful and behaving like a ruderal, August 9, 1938, *David D. Keck 4892* (Dudley Herbarium of Stanford University). Isotypes being distributed to University of California, Carnegie Institution, Gray Herbarium, Kew, New York Botanical Garden, Pomona College, and United States National Herbarium.

First collected near the type locality, at 1430 m. elevation, by Frank

W. Peirson, no. 10297 (CI,[1] SU), in dry soil on meadow borders, July 9, 1932. The following three collections from Lassen County are morphologically indistinguishable from the type collection and so are to be referred to this species: 5 miles south of Likely, on sagebrush-covered hills with scattered junipers, elevation 1400 m., *Keck & Clausen 3729* (CI); Spalding's, Eagle Lake, elevation *ca.* 1585 m., *J. T. Howell 12494* (CAS, Ch, CI); Susanville, among yellow pine, elevation 1280 m., *Keck 949* (CAS, CI, SU). Collections from Duncan Horse Camp, 6 miles east of Perez, *Howell 12268A* (CAS, CI), and Plum Valley, above Davis Creek, Warner Mountains, *Howell 12025* (CAS, Ch, CI), both from Modoc County, appear to be the same.

Madia subspicata Keck sp. nov.

Caule 10–15 cm. alto stricto tenue praecipue simplici vel superne sparsim breve ramoso sicut foliis aliquanto flavoviride piloso viscido-puberulo cum prominenti stipitatis glandulis ornato; foliis linearibus 2–7 cm. longis usque ad 3 mm. latis; capitulis subspicatis axillaribus quam foliis brevioribus; pedunculis brevissimis; involucro ovato 6–7 mm. alto; corollis disci 5–15; antheris nigris; acheniis radii ca. 3 mm. longis minute striatis purpureo-maculosis cuneatis (lineari-oblongis in sectione); acheniis disci similibus. $n = 8$.

Type, from the mouth of Wildcat Canyon, Stanislaus River, near Knights Ferry, Stanislaus County, California, Sec. 31, T. 1 S., R. 12 E., at 50 m. elevation, June 2, 1933, in fruit, *David D. Keck 2453* (Dudley Herbarium of Stanford University); isotypes, Carnegie Institution, Pomona College.

Other collections referable to this species are all from California, as follows: 3 miles southwest of Richardson Springs, along Mud Creek, Butte County, 150 m. elevation, May 30, 1933, *Keck 2404* (CI, Po, SU); Chico Creek, 5 miles east of Chico, Butte County, May 17, 1915, *Heller 11878* (CAS, Ch, GH, M, NY, OSC, SU, US); Sec. 15, T. 2 N., R. 11 E. (east of Milton, Calaveras County), 395 m. elevation, *Roseberry 207* (V); Bear Creek, Tuolumne County, 320 m. elevation, May 14, 1919, *Williamson 78*, in part (CAS, Po, RM, SU); Mormon Bar, Mariposa County, May 28, 1895, *Congdon* (Minn).

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[1] The abbreviations within parentheses on this page refer to the following United States herbaria: CAS, California Academy of Sciences; Ch, Chicago Natural History Museum; CI, Carnegie Institution; GH, Gray Herbarium, Harvard University; M, Missouri Botanical Garden; Minn, University of Minnesota; NY, New York Botanical Garden; OSC, Oregon State College; Po, Pomona College; RM, Rocky Mountain Herbarium, University of Wyoming; SU, Dudley Herbarium, Stanford University; US, United States National Herbarium; V, Vegetation Type Map Herbarium, University of California.

IV

LAYIA PENTAGLOSSA

Members of the genus *Layia* are prominent among the lowland plants that contribute color to the California landscapes in springtime. All fourteen members of the genus are found in this state; twelve occur nowhere else, and half a dozen are narrow endemics. Cytologically, this assemblage falls into two natural groups: one, of six species, each with 7 pairs of chromosomes; and the other, a group of eight species with 8 and 16 pairs. Only one species of the latter group is tetraploid.

The cytogenetic relationships of the species of *Layia* have been studied intensively for a number of years and are now quite thoroughly understood. A résumé of this information has been recorded elsewhere (Clausen, Keck, and Hiesey, 1941), and a complete account of these investigations is in preparation. Suffice it to say here that all species of the 7-chromosome group can be interlinked by partially fertile hybrids, and that five species of the 8-chromosome group can be similarly interlinked. Three members of the latter group have no close relatives and produce hybrids only with great difficulty or not at all. There is, also, a wide genetic barrier between the 7-chromosome and the 8-chromosome Layias. They are very difficult to intercross, and in the few instances where hybrids are obtained these are sterile except when their genomes are added together to form an amphiploid, like *Layia pentaglossa* described here. Hence *Layia pentachaeta* and *L. platyglossa*, its parents, are members of distinct cenospecies.

The parental species. *Layia pentachaeta* A. Gray is an 8-chromosome species found principally in the wooded foothills along the western flank of the Sierra Nevada from Placer County southward to the Tehachapi Mountains and thence through the foothills around the head of the San Joaquin Valley as far north as western Fresno County. Its distribution is shown on the map, figure 46. Typical *pentachaeta*, from the Sierra Nevada foothills, is yellow-rayed. The subspecies found at the head of the San Joaquin Valley and in the inner Coast Range is white-rayed and one of the most showy plants in the genus. It was a plant of the latter form from Caliente Creek, Kern County, which was used as the maternal parent of the experimentally produced amphiploid.

The paternal parent was a member of the inland ecotype of *Layia platyglossa* (F. et M.) A. Gray, a 7-chromosome species. This species is

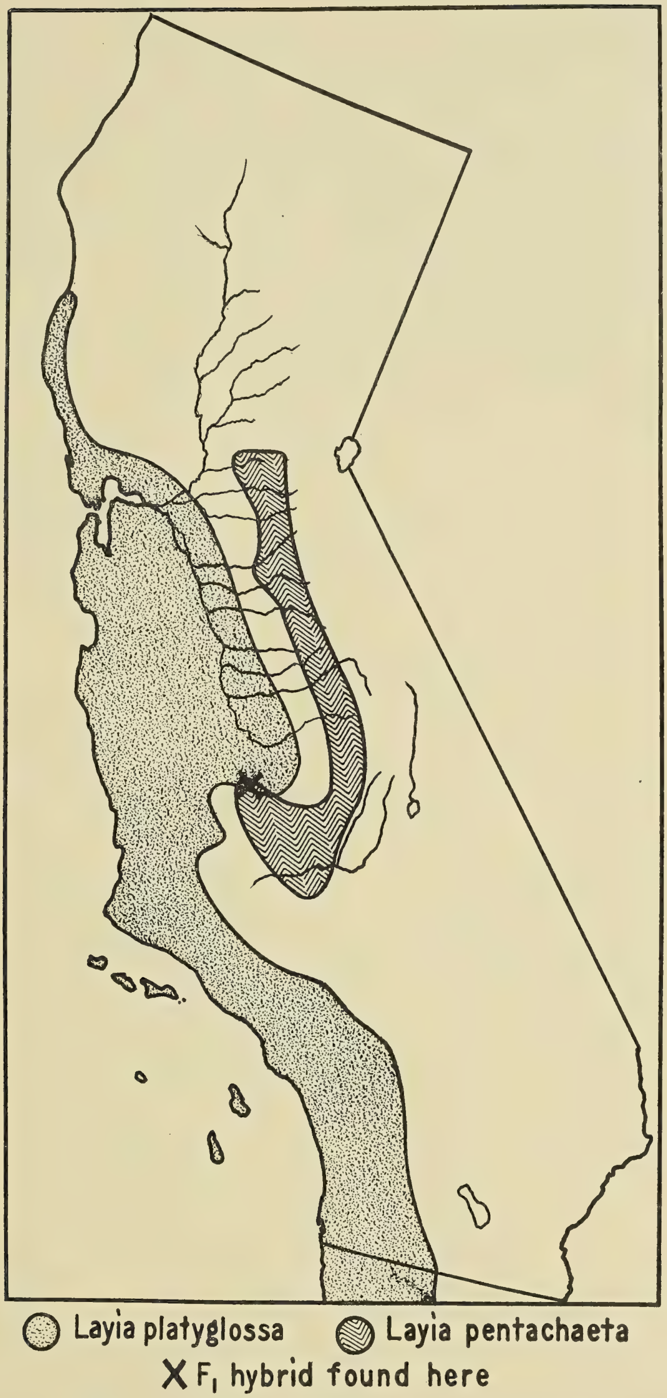


Fig. 46. Distribution of *Layia pentachaeta* and *L. platyglossa* in California

abundant on the grasslands on the plains and hills of coastal California from Mendocino County southward to northern Baja California, but it is infrequent in the interior valleys. The map shows that the distributions of the two species scarcely overlap, and then only at one point in the inner South Coast Range. In *platyglossa* the ray florets are deep yellow with white tips, hence the common name "tidy tips," but a few strains differ in having entirely yellow rays. A plant of this form, from Jolon, Monterey County, was chosen for crossing with the white form of *pentachaeta* in order to afford the greater contrast. This strain happens to have an unusually low fertility, although this fact does not prevent it from being successful and abundant in its native habitat.

The parental species differ by more than a dozen morphological characters in addition to their differences in chromosome number, ecological preferences, and geographical range. A tabulation of their differences and of the character combination found in their amphiploid offspring is given in table 8. The F_1 hybrid was similar to the amphiploid *pentaglossa* except as to its chromosome number and its basal leaves, which were intermediate. Representatives of *Layia pentachaeta* and *L. platyglossa* are shown in figure 47.

TABLE 8

MORPHOLOGICAL DIFFERENCES BETWEEN LAYIA PENTACHAETA, L. PLATYGLOSSA, AND THEIR AMPHIPLOID, L. PENTAGLOSSA

Character	White-rayed *L. pentachaeta*	*L. pentaglossa*	Yellow-rayed *L. platyglossa*
Central stem	long	intermediate	short
Height	45–65 cm.	variable, 23–59 cm.	20–35 cm.
Herbage	fragrant	fragrant	nonfragrant
Basal leaves	blade wide, lobes elongate, toothed	variable (ranging between parents)	blade narrow, lobes short, entire
Involucral bracts	acuminate	intermediate	obtuse
Ligules	white	light yellow, bleaching	deep yellow
Disk florets	orange	orange	yellow
Anthers	yellow	yellow	black
Pappus	15–20 bristles, narrow, white, plumose	17–25 bristles, variable, white or tawny, ± plumose	25–33 bristles, wider, tawny white, nonplumose
Chromosome number	$n = 8$	$n = 15$	$n = 7$

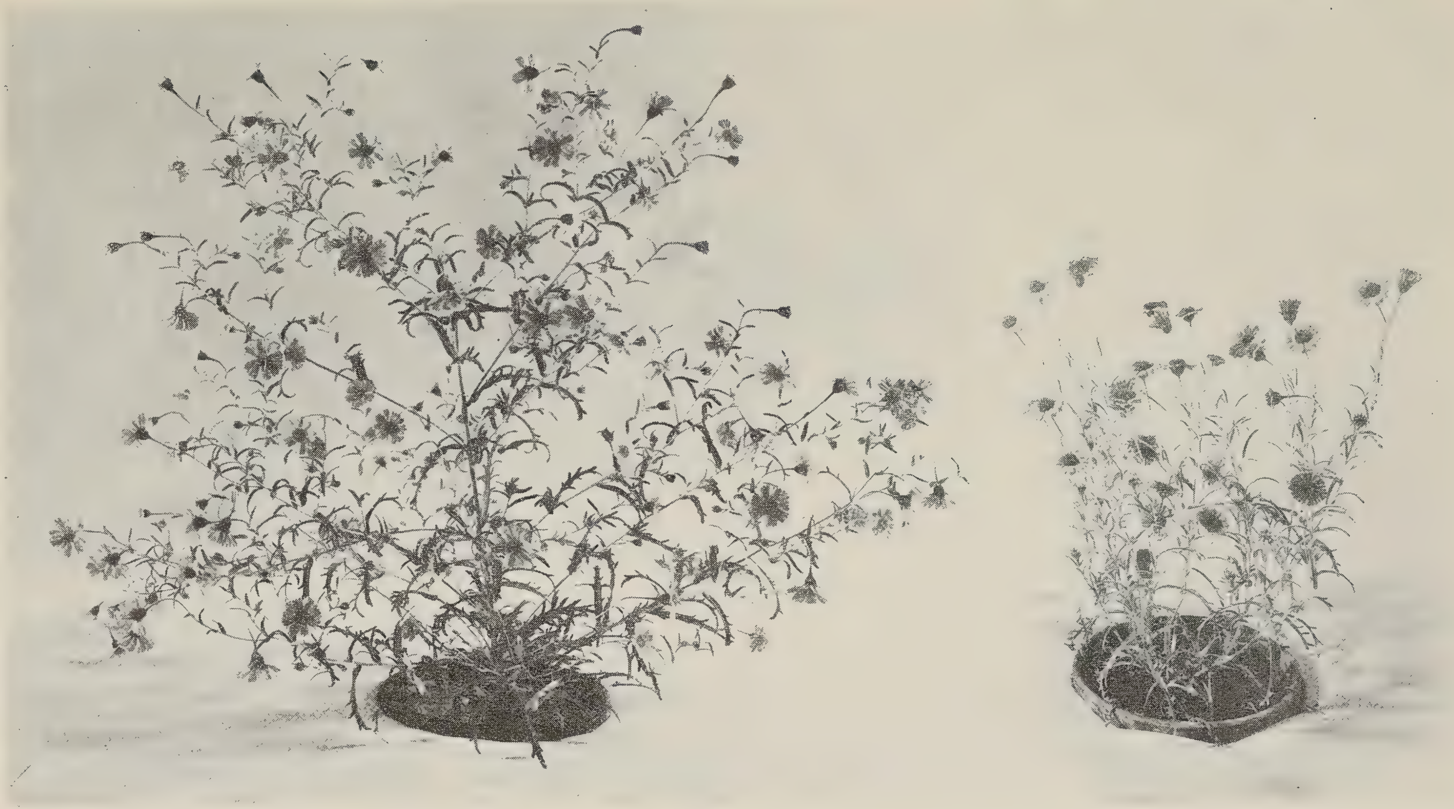


FIG. 47. *Layia pentachaeta* (left) and *L. platyglossa* (right)

SYNTHESIS OF THE AMPHIPLOID

Since both *Layia pentachaeta* and *L. platyglossa* are self-incompatible, they were crossed without previous emasculation in a cage in a screened greenhouse. From four heads on *platyglossa*, 104 well filled akenes were harvested, but none germinated. From seven heads of the reciprocal combination only 35 well developed akenes were found among hundreds of empty ones, but from these 22 hybrids were obtained. The low number of first-generation hybrids is a typical result of crossing members of distinct cenospecies. Several hundred hybrids would normally result from crossing a like number of heads of two species belonging to the same cenospecies. In the pedigree chart, figure 48, this F_1 generation is indicated as culture 2554.

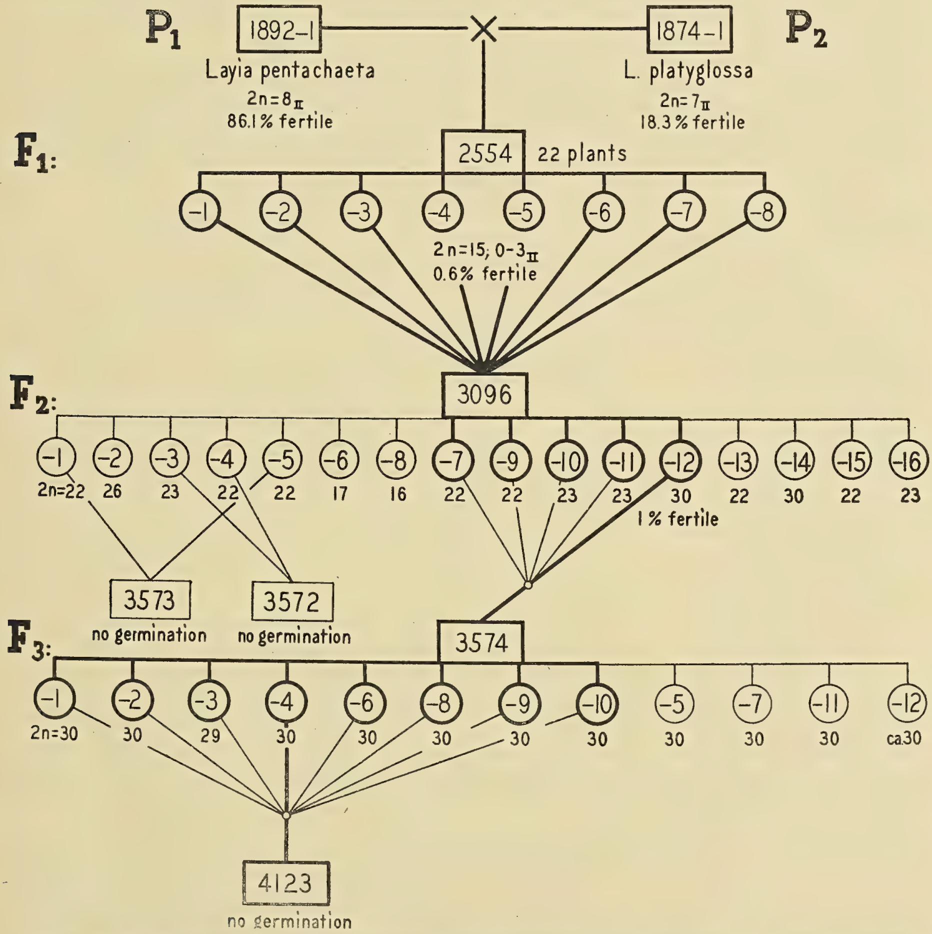


FIG. 48. Pedigree of *Layia pentaglossa*, showing parentage, number of plants, chromosome numbers, and fertilities.

This hybrid population consisted of low, bushy plants considerably shorter than the *pentachaeta* parent and no taller than the *platyglossa* parent. Their most striking feature was the flower color, for the ligules were light yellow at anthesis but soon began to bleach until they were almost pure white. In many other ways the hybrids combined the characters of the parents, although the *pentachaeta* influence was more obvious. Their chromosome number was determined to be $2n = 15$.

Eight of the F_1 plants were placed in an isolation plot in the garden for mutual pollination by insects. The hybrids proved to be very highly sterile, for among the approximately 10,000 akenes possible on eight plants, only 75 seemingly fertile ones were found. The fertility of a sample from all the F_1 plants in the garden was computed to be but 0.6 per cent. When the 75 akenes were sown, however, they produced a second generation of only sixteen plants (3096 of fig. 48), so the effective fertility of these akenes was only 0.12 per cent.

A count of the chromosome numbers in the root tips revealed that the second generation consisted mainly of diploid, triploid, and tetraploid plants, as is shown in table 9. From this table it might appear that plants bearing full sets of parental chromosomes had the best chances for survival, with triploid combinations especially numerous. However, among the triploid plants ($2n = 22$ and 23) there was a great deal of morphological variation, indicating much recombination of the characters of the two parent species.

TABLE 9

SOMATIC CHROMOSOME NUMBERS IN F_2 PROGENY OF LAYIA PENTACHAETA, $n = 8$, × L. PLATYGLOSSA, $n = 7$

(Diploid, triploid, and tetraploid levels are indicated by boldface type)

Chromosome numbers, 2*n*	Number of plants	Chromosome numbers, 2*n*	Number of plants
16	1	24	0
17	1	25	0
18	0	26	1
19	0	27	0
20	0	28	0
21	0	29	0
22	7	**30**	2
23	4		—
		Total	16

The nature of the character recombination in the F_2 progeny is indicated in table 10. In this table an attempt has been made to symbolize the variation, for the sake of simplicity. Eight differences between the parent species were selected for tabulation. These are listed in the legend of the table. When a character of the hybrid resembled that of the *pentachaeta* parent, a value of 1 was assigned; if the resemblance was definitely on the *platyglossa* side, the value 3 was given; but if the character was intermediate, the value 2. Thus a plant like *pentachaeta* in all eight characters receives the formula 11111111, and one like *platyglossa* is 33333333. A plant intermediate in all eight characters would be 22222222. The index values given in the last column in table 10 represent the sums of these numbers. The chromosomal situation in these plants is also presented in this table.

TABLE 10

Layia pentachaeta × L. platyglossa F_2, number and pairing of chromosomes and combinations of eight characters

(The characters are indicated by numerals in terms of resemblance to parental species [see text], and are listed in the following order: basal leaves; color of ligule, of disk, and of anther; number, width, pubescence, and color of pappus [cf. table 8].)

Plant	Chromosomes			Polyploidy	Characters	
	2*n*	Number of			Combination	Index value
		Pairs	Singles			
L. pentachaeta	16	8	none	2n	11111111	8
L. platyglossa	14	7	none	2n	33333333	24
F_1	15	1–3	13–9	2n	22112112	12
F_2:						
3096-1	22	7–8	8–6	3n	12122131	13
-2	26	11–12	4–2	irregular	22313113	16
-3	23	5–6	13–11	3n	12112113	12
-4	22	7–10	8–2	3n	12331111	13
-5	22	6–8	10–6	3n	12213111	12
-6	17	7	3	irregular	12313133	17
-7	22	4–7	14–8	3n	22113111	12
-8	16	7–8	2–0	2n	32332133	20
-9	22	6–10	10–2	3n	22122211	13
-10	23	7–10	9–3	3n	11111211	9
-11	23	3–10	17–3	3n	32111312	14
-12	30	15(–14)	0(–2)	4n	11212212	12
-13	22	5–7	12–8	3n	31311211	13
-14	30	15(–14)	0(–2)	4n	22123112	14
-15	22	7–8	8–6	3n	32213123	17
-16	23	7–10	9–3	3n	12211212	12

The recombination of characters in the F_2 plants is much more complete than one would expect from the fact that the chromosome numbers fell mainly into three levels of polyploidy. For example, one might expect a plant like 3096-8, having $2n = 16$ chromosomes, to be pure *pentachaeta*, but this plant is morphologically the most like *platyglossa* of any in the population, with a character index value of 20. Similarly, the index values of 22-chromosome triploids vary between 12 and 17, and of 23-chromosome ones between 9 and 14. This variation would appear to be impossible if the triploids contained basically the same genomes.

The recombination of characters in the F_2 progeny indicates that their classification as diploid or triploid is not correct in the strict sense that they contain intact sets of parental genomes. On the contrary, they appear to have received recombined genomes, in which the chromosomes may have been partially shuffled, or in which an occasional gene exchange between nonhomologous chromosomes may have taken place. The presence of plants having 17 and 26 chromosomes further proves that partial sets of chromosomes can produce viable offspring here. The morphological variability in this F_2 is therefore in marked contrast with the relative uniformity in the F_2 progenies of the *Madia nutrammii* and *M. citrigracilis* lineages.

The plants of the F_2 generation were, on the average, slightly more vigorous than those of the F_1, although a few individuals, such as 3096-8, -13, and -15, were dwarfish and subnormal. None had the long central stem characteristic of *pentachaeta*, and the branching of most plants resembled that of *platyglossa*. In some the side branches dropped off easily as though an abscission layer had been formed in their axils, and this particular character persisted in the F_3.

The fertility of the F_2 generation was very low. Some apparently good akenes developed in most plants, but when these were sown they either did not germinate or, at best, germinated very poorly. Upon re-examination it was found that many akenes with developed pericarps contained embryos that had aborted at an early stage of development.

Six triploids were paired together in cages in a screened greenhouse for mutual pollination. The first two pairs, 3096-1 and -5, and -3 and -4, developed some apparently good seeds, but these did not germinate. The third pair, 3096-7 and -11, produced no good akenes in the greenhouse in the first part of the season, so they were moved to a garden isolation plot for open pollination with 3096-9, -10, and -12 (see fig. 48). This combination of four triploids and one tetraploid produced some apparently good akenes on each plant, although mainly on the tetraploid (3096-12).

An F_3 population of twelve plants (3574 of fig. 48) was produced from 126 apparently good akenes harvested on the tetraploid F_2 plant. Eleven of these F_3 plants were tetraploid with $2n = 30$ chromosomes, and one was near tetraploid with $2n = 29$. Only one or two good akenes per plant were obtained from the triploid F_2 individuals in the isolation plot, and these were not sown.

The twelve F_3 plants showed much variation in size, three or four approaching the *pentachaeta* parent, and others being weak and small. All were uniform in regard to certain characters but variable for others, as is indicated under *pentaglossa* in table 8. The amphiploid *Layia pentaglossa* is represented only by this F_3 population and the two 30-chromosome plants in F_2.

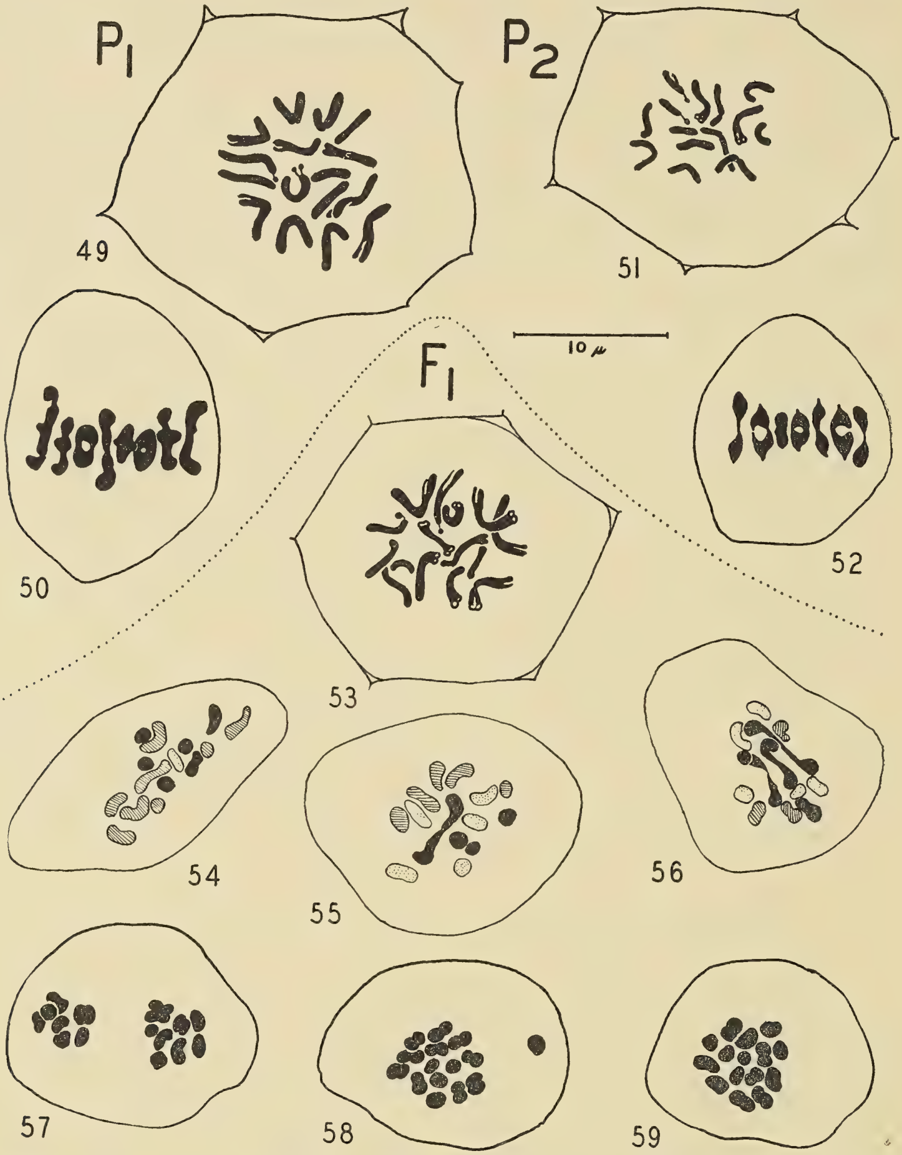
From the chromosome numbers it appears that all F_3 offspring resulted from the pollination of the tetraploid mother plant by diploid pollen from the triploids. Specifically, it was mainly the diploid pollen with 15 chromosomes, rather than that with 14 (double *platyglossa*) or 16 (double *pentachaeta*), that was successful. Since the plants were all apparent amphiploids, it is rather surprising that considerable variation and recombination of characters continued in the third generation.

The fertility was not improved in the F_3. The eight plants indicated in the pedigree (fig. 48) were isolated in the garden for open pollination, but they were highly sterile although they flowered freely. One (3574-4 in fig. 48) produced approximately 600 akenes, an apparent fertility of 12 per cent, but none of these germinated when sown. The apparent fertilities of the other plants were negligible. This terminated the line of *Layia pentaglossa*, which both in vigor and in fertility was inferior to the two amphiploids of *Madia* described above.

CYTOLOGICAL BASIS OF LAYIA PENTAGLOSSA

The race of *Layia pentachaeta* from Caliente Creek, with $2n = 16$, and that of *platyglossa* from Jolon, with $2n = 14$, like the other races studied of their respective species, are very regular cytologically. Typical somatic and meiotic chromosome plates of the *pentachaeta* parent are shown in figures 49 and 50, and of the *platyglossa* parent in figures 51 and 52. The chromosomes of these species differ perceptibly in size, those of *pentachaeta* being the larger. This makes it possible to identify the largest chromosomes in their hybrids as having originated from the *pentachaeta* parent, and the smallest as having come from *platyglossa*, but those of intermediate size cannot be satisfactorily identified.

Behavior of chromosomes in the F_1. Figures 53 to 59 illustrate the chromosomal behavior of the first-generation hybrid. In the somatic plate, figure 53, it is possible to recognize the parentage of some of the chromosomes. The meiotic figures were all from one floret, those of first metaphase (figs. 54–56) coming from one anther lobe, and those of second metaphase (figs. 57–59) from another. The cell of figure 59 has



Figs. 49–59. Somatic and meiotic chromosome plates of *Layia pentachaeta* (P_1), *L. platyglossa* (P_2), and their F_1 hybrid. For details, see text. × 2000.

the appearance of a first metaphase with 15 pairs, but the fact that it was found adjacent to the typical second metaphase of figure 57 reveals that it corresponds to that stage.

The chromosomes of *pentachaeta* and *platyglossa* are not very homologous, for few or no pairs are formed during first metaphase in the F_1 generation. Two plants were investigated, one of which had an average of 2.4 pairs per cell, and the other 0.8 pairs, as shown in the left half of table 11. Clear examples of heteromorphic pairs were not obtained, although such might be expected in this material.

TABLE 11

Behavior of chromosomes in meiosis of pollen mother cells of Layia pentachaeta × L. platyglossa F_1

Pairing, metaphase I	Number of cells Plant 2554-1	Number of cells Plant 2554-2	Distribution, metaphase II	Number of cells Plant 2554-1	Number of cells Plant 2554-2
15_{I}	4	16	15 + 0	5	28
$1_{\text{II}} + 13_{\text{I}}$	12	12	14 + 1	1	5
$2_{\text{II}} + 11_{\text{I}}$	26	7	13 + 2	0	2
$3_{\text{II}} + 9_{\text{I}}$	21	1	12 + 3	2	2
$4_{\text{II}} + 7_{\text{I}}$	13	0	11 + 4	1	4
$5_{\text{II}} + 5_{\text{I}}$	2	0	10 + 5	8	1
	—	—	9 + 6	6	3
Total cells	78	36	8 + 7	8	3
Mean pairs	2.4	0.8		—	—
			Total nuclei	57	68
			Diploid nuclei (%)	8.8	41.2

In cells where pairs were formed, the univalents appeared to move to the daughter nuclei during the first division, for stray chromosomes were rarely observed in the subsequent second metaphase. The distribution of the chromosomes as revealed by second metaphases is tabulated in the right half of table 11, and examples are shown in figures 57 to 59. Single chromosomes were often lost in the cytoplasm (fig. 58), and irregular tetrads, supernumerary microspore groups, and micropollen were commonly observed.

In cells with no pairing the chromosomes did not appear to disjoin during first metaphase, so that at second metaphase only one nucleus was found. This was diploid, containing the 15 chromosomes of both parents (fig. 59). Only one division, an equational one, took place in these cells, resulting in the formation of dyads. The correlation between the chromosome distribution during meiosis and the formation of gametes is illustrated by the plant 2554-2, in which 41.2 per cent diploid nuclei were observed in second metaphase (table 11), and 38.9 per cent dyads among its tetrads.

In hybrids between species as distantly related as *pentachaeta* and *platyglossa*, in which the chromosomes show very little affinity, the pairing is quite variable within pollen mother cells of the same plant. Also, sister plants may differ appreciably in the degree of chromosome pairing, as is shown by the two plants tabulated in table 11. Probably the behavior of the chromosomes in such hybrids may be influenced by highly localized physiological conditions which may shift the delicately balanced meiotic process in one direction or another.

Behavior of chromosomes in the F_2. If the triploids contained only pure genomes of the parent species, one would expect 7 pairs plus 8 singles in the 22-chromosome plants, and 8 pairs plus 7 singles in those with 23 chromosomes. Very few triploids conformed to this pattern, for most of them were capable of producing more pairs than would be expected, as table 10 shows. This suggests that the sex cells producing them were neither strictly haploid nor diploid.

The cytological situation in six selected plants of this F_2 population is illustrated in the two groups of figures, 60 to 67 and 68 to 73. These plants are listed below and their chromosomal situation is compared with their character combinations, indexed in table 10.

3096-1 (figs. 60, 61), $2n = 22$. The 8 singles were large, and the pairing was that of a true triploid; it is therefore likely that this plant of intermediate morphology had two sets of *platyglossa* chromosomes and one of *pentachaeta*.

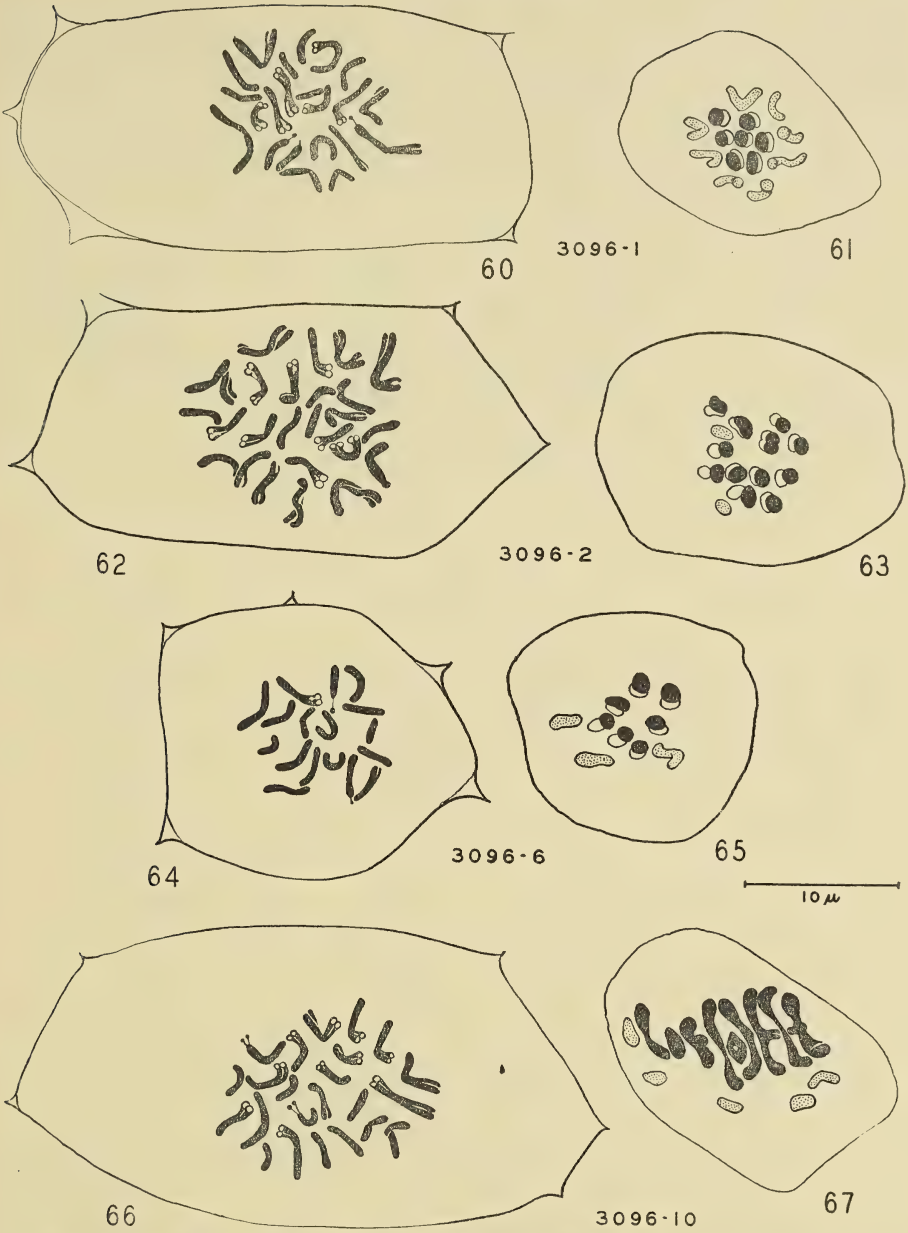
3096-2 (figs. 62, 63), $2n = 26$. The 12 pairs consisted of large and small chromosomes, and the 2 singles were small. This plant approached the tetraploid condition, but in morphological characters it resembled *platyglossa*, so that it probably lacked some *pentachaeta* chromosomes.

3096-6 (figs. 64, 65), $2n = 17$. This plant was regularly 7-paired, its 3 singles were fairly large, and in morphological characters it approached *platyglossa*. Therefore, its 7 pairs were probably from *platyglossa*, and the 3 singles from *pentachaeta*.

3096-10 (figs. 66, 67), $2n = 23$. This plant had up to 10 pairs, with corresponding numbers of singles. Figure 67 suggests that at least 5 and possibly 7 of the larger pairs were from *pentachaeta*, with 2 or 3 small pairs from *platyglossa*. The 5 singles were small to medium-sized. The morphological characters of this plant were mostly those of *pentachaeta*, in conformity with its large number of *pentachaeta* pairs. The most interesting fact about this "triploid" is that it appears to have a mixture of pairs from both parental species.

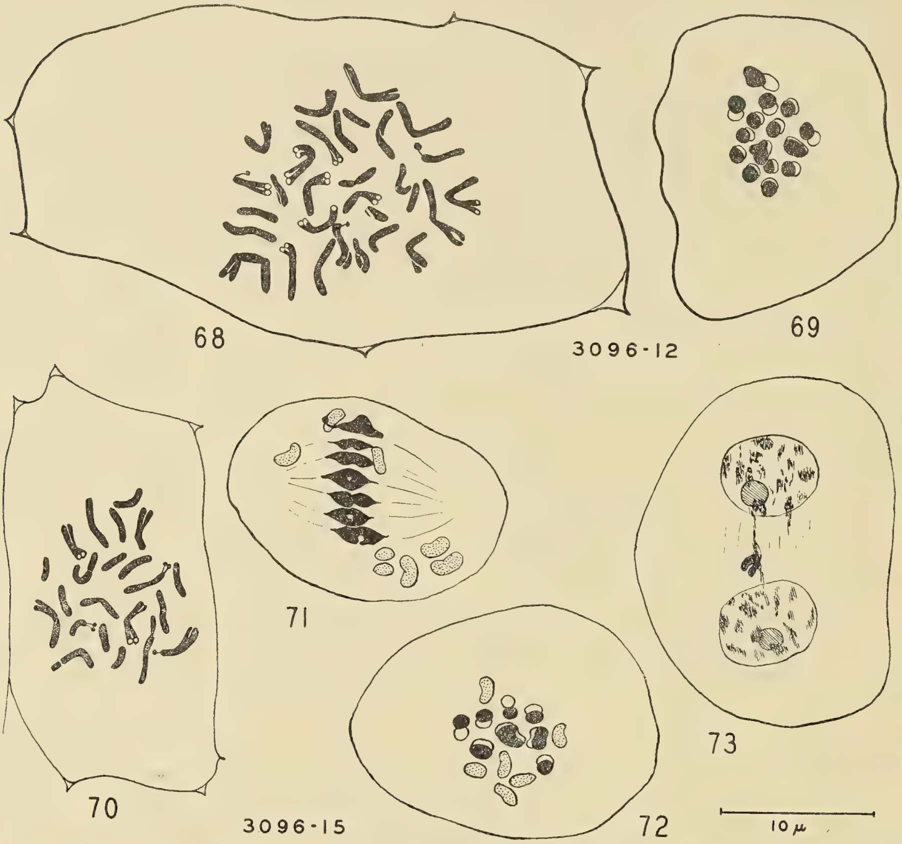
3096-12 (figs. 68, 69), $2n = 30$. This amphiploid, with 15 regular pairs of chromosomes, had large, small, and medium pairs in both the meiotic and the somatic figures. In morphological characters it approached, but did not duplicate, the F_1.

3096-15 (figs. 70–73), $2n = 22$. Although this plant behaved like a triploid with 7 or 8 pairs of chromosomes, the pairs and singles appeared to include both large and small chromosomes. Morphologically this plant approached *platyglossa*. Although numerically a triploid, it probably contained repatterned parental genomes. One to three chromatic bridges (fig. 73) were often observed to remain until second metaphase, suggesting that even some of the chromosomes themselves had been repatterned.



FIGS. 60–67. Somatic and meiotic chromosome plates of four plants of *Layia pentachaeta* × *L. platyglossa* F_2. Univalent chromosomes are stippled. Details in text. × 2000.

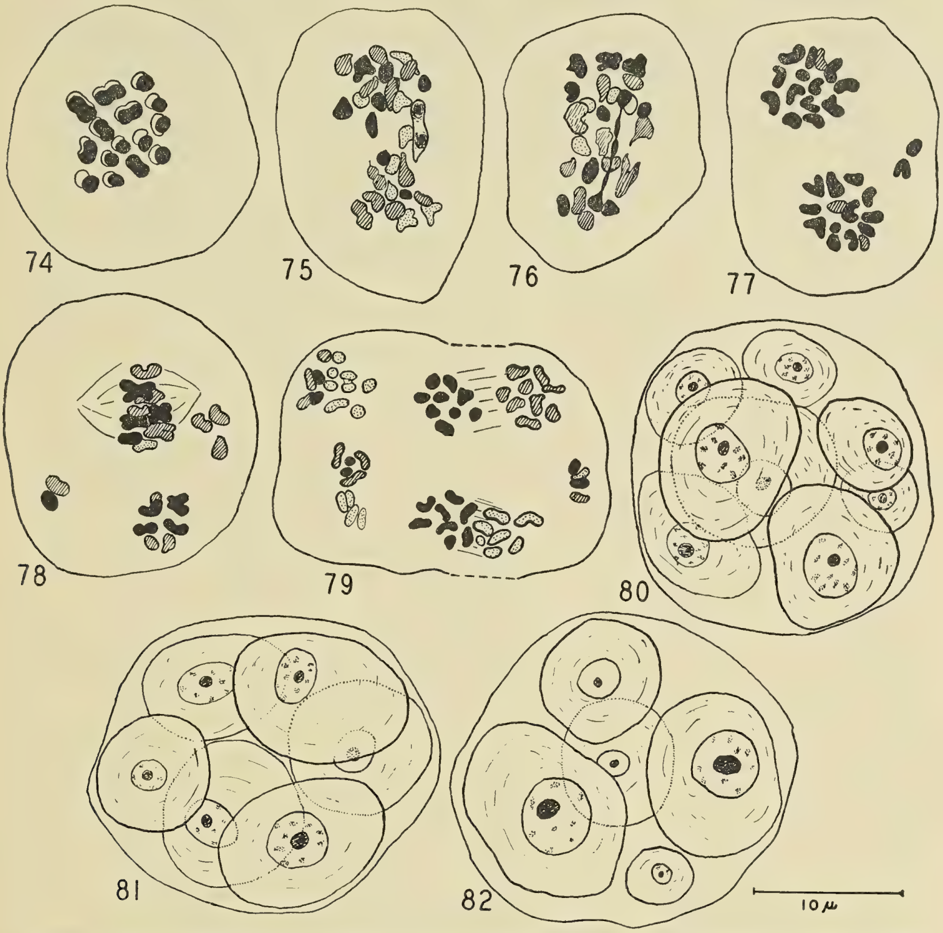
The weak pairing in F_1 would suggest that crossing over was of slight consequence. There is also very little evidence of structural chromosomal rearrangements, for chromatin bridges were clearly seen only in 3096-15. Indeed, chromatin bridges are very rarely found in any of the hybrids of *Layia*. The evidence, therefore, points to a limited exchange of whole chromosomes between the sets of *pentachaeta* and *platyglossa* as the main cause of the variation in the F_2 and of the pairings observed. As will be brought out in later discussion, the instability of *pentaglossa* is probably caused by this exchange of specifically distinct chromosomes.



FIGS. 68–73. Somatic and meiotic chromosome plates of two additional F_2 plants. Univalents stippled. Details in text. × 2000.

MEIOTIC DEGENERATION IN THE F_3. Pairing between the chromosomes was strikingly more regular in the F_3 than in the F_2, as one might expect from the fact that eleven of the twelve F_3 plants were amphiploid with $2n = 30$ chromosomes. Almost all these plants had 15 pairs in some cells (fig. 74) and 14 pairs and 2 singles in others. In the 29-chromosome plant there were 1 or 3 singles. A dwarf, weak plant, 3574-12, varied in having from 15 to 9 pairs plus a corresponding number of singles. No indications of secondary association were found, although such might be expected if one or two of the pairs were duplicated.

The apparent cytological regularity of first metaphase did not continue, for even by first anaphase abnormal distribution could be observed. Figures 75 and 76 show side views of first anaphases with chromosomes delayed in their movement toward the poles. The apparent bridge between two of the chromosomes in figure 76 may be due to a delayed separation of one of the long *pentachaeta* pairs, for such bridges were not accompanied by fragmentations, nor did they persist until the second division.



FIGS. 74–82. Chromosome behavior and distribution in the F_3 generation, *Layia pentaglossa.* Details in text. × 2000.

At second metaphase it was almost invariably found that some of the chromosomes had formed detached groups outside the daughter nuclei (figs. 77, 78). Such a situation gives rise to many nuclei when the divided chromosomes distribute in second anaphase. Figure 79 shows a cell in second anaphase having eight groups of chromosomes in which a total of 59 of the expected 60 are visible; there are from 4 to 13 chromosomes in each nuclear group of this typical pollen mother cell. At the tetrad stage these irregularities resulted in very variable microspore groups, some of which are shown in figures 80 to 82. The cells in these groups were very unequal in size, and their number varied between 5 and 10, with 8 most commonly found. It is therefore not surprising that the F_3 plants were sterile.

Behind this upset in the mechanism of the movement of the chromosomes, there probably is some basic disturbance in the genetic balance of *pentaglossa.* The F_3 was more uniform than the F_2 with respect to some external characters, but segregation with respect to others continued in spite of the doubling of the chromosomes. This segregation is illustrated by the variation in characters of basal leaves in the tetraploid *pentaglossa* F_3 population. In figure 83 these may be compared with those of the parental species and of the F_1. Each leaf of the parents and of the F_1 was taken from a different individual, to show the relative uniformity in these cultures. Two basal leaves from each of the first eleven F_3 plants are shown, to illustrate the variation between sister plants as compared with the individual uniformity. The size of these leaves expresses to a certain degree the vigor of the plant.

The basal leaves of *pentachaeta* and *platyglossa* have differences of specific value. In the F_1 they were intermediate and fairly uniform, but in the F_2 and the F_3 they were highly variable. These variations doubtless reflect interspecific exchanges of whole chromosomes, or possibly of genes, and could have been caused by the duplication of one or two pairs of chromosomes of one parent and the absence of a corresponding number of pairs of the other. With the parents as remotely related as they are, a very slight shuffling between their chromosomes is probably sufficient to upset balanced development.

The mere production of a relatively successful F_1 hybrid, followed by a doubling of the chromosome number, is therefore no guarantee of ultimate success of the line. In cases where the chromosome sets of the parent species have already been altered by shuffling in the hybrid, their doubling will not enable the new form to escape the constitutional weaknesses of such interspecific exchanges. Polyploids of this kind are not true amphiploids in the strict sense of the term, for they have not received all the genes from both parental species. According to this interpretation, *Layia pentaglossa* can qualify only as a quasi amphiploid.

True amphiploidy, on the other hand, neatly circumvents the destruc-

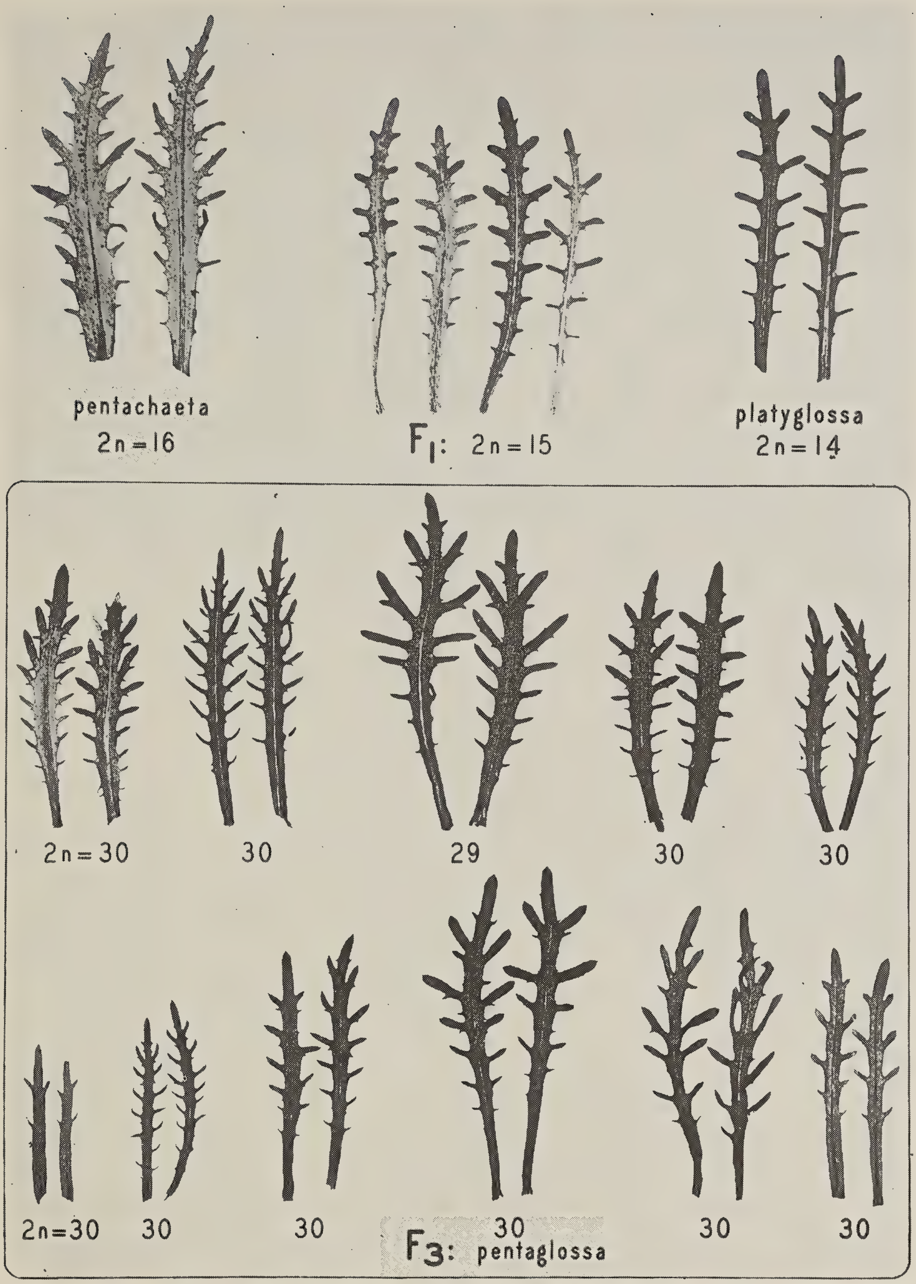


Fig. 83. Basal leaves showing variation in the amphiploid *Layia pentaglossa*. Parent species and the F_1 above, *pentaglossa* below. In the F_3, two basal leaves from each of the first eleven plants (3574-1 to -11 of fig. 48) are shown. In the parents and the F_1, each leaf is from a different individual.

tive effect of interspecific gene exchange through the doubling and preservation of nonhomologous sets of chromosomes.

A NATURAL HYBRID

The distribution map (fig. 46, p. 47) shows an area in the inner Coast Range occupied by both *Layia pentachaeta* and *L. platyglossa*. Actually, within that area only one station is known where the species grow together, for they do not occur continuously, but only as populations scattered here and there. This station is along Cholame Creek, in the southeastern corner of Monterey County, California, and is marked on the map. At this locality a single plant of the white-flowered ecotype of *pentachaeta*, such as was used in the production of *pentaglossa*, was found growing within a population of the common form of *platyglossa*, having yellow rays with white tips. The seed on the *pentachaeta* plant was harvested with the realization that hybridization must have taken place. These seeds produced only three plants, of which one was *pentachaeta*, doubtless a self-pollination induced by the stimulation of the *platyglossa* pollen, and the other two were the expected hybrids. These were recognized both by their morphological characters and by their chromosome number, $2n = 15$. One of the hybrids was kept in the screened greenhouse and the other in the garden. The garden plant had an opportunity to receive pollen from several strains of both parental species and from many other kinds of *Layia*, but neither hybrid set any good akenes.

Chromosomal pairing in these two hybrids was strikingly similar to that found in the artificial hybrids obtained from quite different strains. The same very reduced pairing in the first meiotic division was observed, as well as similar irregularities in later stages. In the greenhouse plant, four unreduced, diploid sex cells were found that were similar to those of the artificial F_1 hybrids.

The presence of diploid gametes in this natural hybrid suggests the possibility that the amphiploid *Layia pentaglossa* could occur in the wild, and perhaps countless trials have already been made. The infrequency of the tetraploids in the F_2 would require that many F_1 hybrids arise simultaneously in one population to assure the production of at least two of them. Mathematically this is a remote possibility, and since unbalanced quasi amphiploids are likely to result from this cross, the chances of success appear very slight, even if the combination should arise.

V

BIOSYSTEMATIC RELATIONSHIPS AND AMPHIPLOIDY

The findings reported here represent a small part of a series of investigations designed to clarify current concepts regarding the organization of living things and their mode of evolution. This objective has been approached from the point of view of classical taxonomy, including morphology and distribution, as well as from the points of view of ecology, genetics, and cytology. It is therefore appropriate to examine the findings on amphiploidy in the perspective of the entire series of investigations.

PRINCIPLES GOVERNING BIOSYSTEMATIC INVESTIGATIONS. Experimental studies reveal an organization of living things based primarily upon genetic and ecologic principles. The genetic principles include those of the chromosomes, and the ecologic ones include that of physiologic fitness to the environment in which the organism is native. Genetic and ecologic factors control the morphological characters utilized in separating species and other natural units. Therefore, once this relation has been determined by experiment, these morphological characters can be successfully employed to trace the distribution of the species in the wild, and they become indicators of more basic genetic-physiologic differences.

Two fundamental principles govern the appearance and distribution of wild plants and probably of all living things, as follows:

1. Natural species consist of individuals whose genes are in internal balance so that a harmonious physiologic and morphologic development is assured generation after generation.

2. The individuals of wild species not only are balanced internally, but fit their natural environment; they are in rhythm with the seasons and adapted to the over-all conditions of temperature, moisture, wind, soil, light, and biotic elements. Individuals out of balance with their environment are unable to compete with others better fitted.

Considering the number of genes involved in the differentiation of kinds of plants and the intricacy of their balances, it is not remarkable that it is difficult to restore the balance once it has been broken through a rearrangement of genes following interspecific hybridization. The very intricacy of the interreactions of many genes—largely precluding as they do most of the possibilities of change—may account for the relative stability found in species, many of which have preserved their identity essentially unchanged through geologic periods of time. When species are closely enough related to permit the production of interspecific hybrids, the offspring are characterized by various combinations of gametic and zygotic sterility, poor germination, sublethal seedlings, and an assortment of subnormal plants. These effects are evidently closely related and indicate very severe disturbances in the gene-determined physiological balances.

It is therefore natural that species, which have to meet the exacting requirements of both the internal and external balances, change slowly, and that they are relatively few considering the large number of genes available. It is more remarkable that widely different subspecies and ecotypes of one species from geographically and climatically widely separated areas are able to interchange their genes freely without consequential disturbances in the intricate internal balance.

THE BIOSYSTEMATIC UNITS. From an abundance of experimental data it is now clear that natural units of various ranks exist. The higher units are separated by more distinct internal barriers than the lower units within them, hence they are more effectively prevented from exchanging genes. This is taken to indicate that the genetic balances among the units of higher rank are the more unlike. Many small and gradual steps are involved in the differentiation of the natural units from the local population to the genus. One therefore finds natural units on different levels, the better marked of which have received special recognition. These represent significant nodes, or departures, in evolutionary differentiation. Four major levels are recognized in the present experimental investigations. These are the foundation of a classification which is basically biological rather than morphological. Three of the biological units recognized are adopted from Turesson (1922*a*, 1922*b*), and the fourth and most inclusive from Danser (1929). This classification is a further development of one previously proposed by the writers (1939). These biological—or, better, biosystematic—units are as follows:

1. *Ecotype*. Species that occupy a series of contrasting environments develop genetically and physiologically distinct ecologic races, the ecotypes, which are suited to these environments. Ecotypes of one species have the same internal balance, for there is no genetic obstacle to a free interchange of their genes when they meet and hybridize. Each of such ecotypes, however, strikes a different balance with the environment and is prevented from free migration to other environments by natural selection.

Some species are monotypic, that is, they contain only one ecotype.

Then they occupy only one environmental zone, although they may have a wide geographical distribution within a climatically fairly uniform area. Also, some so-called species, not separated by genetic barriers but recognized only by morphology and ecology, are but ecotypes of one species. The ecotype is a biological unit defined in terms of ecology and genetics combined, but taxonomically it frequently approximates the geographical subspecies.

2. *Ecospecies.* Different species have evolved separate genetic systems that are balanced both internally and externally. These balances are so intricate that the genes of related systems cannot be freely interchanged without seriously impairing the ensuing development of the offspring. Such constitutional barriers between species are based in the genic structure, but are expressed through physiological development or morphology. These constitutional barriers are carried along wherever a species migrates. This situation differs from that in ecotypes, which depend exclusively for their separation upon their fitness to given environments. The species is thereby insured more permanence than the ecotype.

Species capable of a limited interchange of genes with one another are ecospecies of one cenospecies. Like ecotypes of one species, they usually occupy a series of different environments. The ecospecies approximates the species of moderately conservative taxonomists working along conventional lines.

3. *Cenospecies.* Species entirely unable to exchange genes with one another belong to different cenospecies. Their genetic balances have become so unlike that no interchange is possible, although sterile hybrids may at times be formed between them. It is still possible, however, to add all the chromosomes of members of distinct but related cenospecies by the process of amphiploidy. Some cenospecies are monotypic, that is, they consist of only one ecospecies. The evolutionary possibilities open to such cenospecies are few. In most genera the level of the cenospecies approximates that of the taxonomic section or of the species complex, but in small genera it may equal the entire genus. The monotypic cenospecies is usually a very distinct taxonomic species.

4. *Comparium.* Distinct cenospecies which are still able to produce first-generation hybrids with one another belong to one comparium. In accordance with the definition of the cenospecies, such hybrids are always sterile except when all the chromosomes of both parents are added together through amphiploidy. Cenospecies which are unable to intercross and produce even a sterile hybrid belong to different com-

paria unless they are linked through intermediaries. At this evolutionary level the genetic balances have differentiated so far that even the addition of all the chromosomes of members of two comparia is impossible.

In many complex groups of plants the comparium corresponds to the genus, but there are also examples of several adjacent genera composing one comparium. In less complex groups which approach a static condition, it may include only a single taxonomic species. The genus *Ginkgo*, for instance, of the monotypic order Ginkgoales, has evidently become depleted in biotypes to such a degree that today it constitutes a single comparium, with one cenospecies, one ecospecies, and possibly only one ecotype.

These four kinds of biosystematic units represent important evolutionary nodes, but many intermediate steps connect them. Briefly, therefore, ecotype differentiation may be considered to represent the evolutionary level at which fitness to more than one major environment evolves. The ecospecies is at that level at which separate units arise through constitutional barriers to successful interbreeding. Beyond the level of the cenospecies gene exchange is impossible, although the addition of all the genes of members of two cenospecies in the same comparium is still possible through amphiploidy. The comparium marks the limit for even this avenue of evolution, because neither exchange nor addition of genes of related comparia is possible.

In general terms one may say that evolution is reticulate from the level of the ecotype to that of the comparium, but beyond that level it is exclusively of the forked type. The comparium therefore represents a very important node in the evolutionary process. In biosystematic studies on plants, the ecotype is the basic ecological unit, the ecospecies is the basic systematic unit, and the cenospecies and comparium are units of major evolutionary significance.

THE EVOLUTIONARY PROCESSES. If the data from a study of hundreds of different hybrids between taxonomic units of various ranks are correctly interpreted, then it appears likely that one kind of biosystematic unit may evolve from another in successive order. The processes of mutation, recombination, and selection provide the machinery for this development. Distinct ecotypes of one species may successively become distinct ecospecies, cenospecies, and comparia. The process of mutation furnishes the raw materials or genes, and the recombining processes repattern them. As these new forms are subjected to the environment, selective processes eliminate the unfit. The balance between the processes of mutation and recombination on the one hand, and of selection on the other, determines whether a group of organisms is on its way up or on its way out.

Processes of evolution not necessarily activated by hybridization include gene mutation, chromosome multiplication (autoploidy), and chromosome repatterning (translocation and inversion). Presumably it should be possible for these processes to occur at all stages in the evolution of a group, even when, like *Ginkgo*, it has become a monotypic comparium.

Evolutionary processes initiated only by hybridization include chromosome exchange, gene interchange, and addition of sets of chromosomes through amphiploidy. These have their best opportunities in comparia consisting of many cenospecies, with many ecospecies and ecotypes. Such a comparium is possibly in its most active and expansive stage of development. Groups of this nature, with their confusing patterns of interrelations, are those which the taxonomists call critical. Under these conditions the gene interchanges gradually repattern the ecotypes and, to a certain extent, the ecospecies also. The addition of complete sets of chromosomes is most effective in hybrids between cenospecies of the same comparium; for hybrids between species with nonhomologous chromosomes produce the most stable combinations. Amphiploidy is a remarkable way of circumventing gene interchange in interspecific hybrids. Thereby the gene balance of the first-generation hybrid is perpetuated, and the new form emerges suddenly.

In comparia that are rich in ecospecies and ecotypes, nonhybrid evolutionary processes also continue. These occur alongside of gene interchanges and additions of sets of chromosomes, and it is even possible that hybridism accentuates their rate of appearance. Accordingly, systematic groups in this stage of development have their greatest evolutionary possibilities. Conversely, as through the ages a comparium becomes depleted and approaches the monotypic condition, its chances for change through hybridization decrease. This situation is well illustrated by the genus *Sequoia*, which at one time encircled the northern hemisphere and must have consisted of several ecospecies and many ecotypes, but today is represented by only two monotypic species in California. It follows from this discussion that the biosystematic approach to classification gives an evaluation of the stage of development reached by the group studied and of its evolutionary possibilities.

APPLICATIONS OF BIOSYSTEMATIC PRINCIPLES. The biosystematic units are explored by experimental methods. They are usually found to correspond fairly closely to taxonomic units based on careful morphologic and distributional studies. A few interesting discrepancies in critical groups of plants, however, indicate the shortcomings of a purely morphological approach to classification.

In chapter II attention was called to *Layia nutans* (Greene) Jepson, which proved to be a *Madia* when tested by experiments. This is an example of the correctives offered by this approach. Similarly, experiments now indicate that another *Madia*, known for years as *Hemizonia Wheeleri* A. Gray, is no more than an ecotype of *Madia elegans* D. Don. These are not very unlike morphologically, each has 8 pairs of chromosomes, their hybrids are fertile, the second generation is vigorous, and their chromosomes are homologous.

Likewise, the complete genetic compatibility of *Zea Mays* L. with *Euchlaena mexicana* Schrad. (Mangelsdorf and Reeves, 1939) indicates that these two "genera" belong to one ecospecies. Recently Reeves and Mangelsdorf (1942), recognizing the close relationship of these plants, have combined them in *Zea* but retained them as separate species. These and the genus *Tripsacum* belong to one comparium.

Another striking discrepancy is found in the agriculturally equally important genera *Triticum*, *Aegilops*, *Agropyron* (including *Haynaldia*), *Secale*, *Elymus*, *Hordeum*, and *Sitanion*. The combined results of the investigations of many authors indicate that these genera belong to one comparium. The chromosome homologies of their species transcend present generic lines. Although the first three genera are widely accepted today, they have, at one time or another, been included under *Triticum*, and from the genetic point of view this has not been without reason. The synonymies and many "intergeneric" hybrids point to their close relationship.

A still more glaring discrepancy is presented by *Lolium perenne* L. and *Festuca pratensis* Huds. (= *F. elatior* L.). These two grasses are placed in different tribes. They hybridize spontaneously, they are slightly interfertile on backcrossing (Jenkin, 1933), and their chromosomes are homologous, for the hybrid has 7 pairs like each parent (Peto, 1933). Accordingly, they belong to one cenospecies. Hybrids link them with the other species of *Lolium* and *Festuca*, which places both genera in the same comparium. The positive evidence of homology between their chromosomes is a forceful reminder of their close evolutionary relationship not lightly to be ignored, and is in line with similarities in their floral morphology reported by Stebbins (in litt.).

These examples of marked inconsistencies between purely morphological and biological classifications are taken from the Gramineae and the

Compositae, two families admittedly characterized by very complex relationships. The intricacies of these relationships sometimes make it necessary for the classifier to use only one or two characters as key indicators of tribal differences. Such a situation in itself suggests that such tribes may be entirely artificial.

The nature of the complexity within these and other families suggests that they are in a very active stage of evolution. Such situations call for a re-examination of the classification on the basis of adequate experimental data. A recent brief summary of the relationships in the genus *Layia* shows the application of some of the biosystematic principles in a fairly critical genus (Clausen, Keck, and Hiesey, 1941).

REQUIREMENTS FOR SUCCESS IN AMPHIPLOIDS. Amphiploidy is now known to play a very significant role in the evolution of higher plants. Our modern cultivated wheats and other crop plants probably originated through it, and doubtless the process will receive greater attention in future plant breeding. It is therefore of economic as well as of scientific importance to consider the principles that govern the development of successful amphiploids.

When amphiploids were first produced it was tacitly assumed that the simple doubling of the chromosome number would, in some miraculous way, render any sterile hybrid fertile and vigorous. Sufficient information has now been gathered on experimental amphiploids to show that many of them are partly sterile or otherwise unsuccessful. This is exactly what one should expect from the biological concept of species as presented above.

The first condition for a successful amphiploid is that the genomes of its parents fit together properly to insure a harmonious development of the F_1. Weak first generations are more likely to result from crossings between the remotest species it is possible to hybridize than from those between species a little more closely related.

If the F_1 hybrid is vigorous and healthy, a successful amphiploid may be produced, but then it must be remembered that the genes of distinct species are not freely interchangeable without detriment to the offspring. If, therefore, some gene exchange takes place before or after the duplication, as happened in *Layia pentaglossa*, the resulting progeny should be expected to show the same weaknesses that would be observed in F_2 populations of such hybrids with undoubled chromosome numbers. Since the balances that determine success or failure are intricate and delicate, they may be upset by slight genetic interchanges. The principles that govern the development of amphiploids may therefore be stated as follows:

1. Successful amphiploids arise from successful interspecific hybrids. It is essential that the parental genomes which enter the first-generation hybrid interact in such a way as to produce a harmonious and vigorous development.

2. If the amphiploid is to remain successful during succeeding generations, the original balance must remain unchanged. The best way to insure such perpetuation is to use species as parents with chromosomes so different that those of one are nonhomologous with those of the other. This prevents interspecific segregation in their amphiploid.

In terms of biosystematic units, an amphiploid is most likely to succeed if its parent species are members of different cenospecies of one comparium. They should be closely enough related to produce a vigorous F_1 hybrid, but remotely enough so that the balance between their combined genomes can be perpetuated.

VI

CLASSIFICATION OF EXPERIMENTAL POLYPLOIDS ON BIOSYSTEMATIC PRINCIPLES

Polyploids in the plant kingdom have been classified as autoploids and amphiploids. Various criteria have been used in the attempt to designate the differences which such a classification implies, but none of the attempts have been fully satisfactory, because they failed to reckon adequately with the complex organization of plant relationships. It therefore appears desirable to attempt a classification on biosystematic principles.

Autoploids may either spring spontaneously from nonhybrid individuals of a given species, or arise from intra-ecospecific hybrids, which, of course, are fully fertile. Amphiploids, on the other hand, are derived only from inter-ecospecific and inter-cenospecific hybrids, and consequently the F_1 hybrids giving rise to them are either partially or wholly sterile.

Amphiploids in the strictest sense are derived from inter-cenospecific hybrids. Between this extreme and that of true autoploids there are possibilities for chromosome doubling in hybrids between plants of intermediate degrees of relationship. Such combinations are generally unstable, because the chromosomes are not homologous enough to be completely interchangeable, but are too closely related to preserve the original genomes intact. One would not expect many products of evolutionary importance to arise from these intermediate cases. The occurrence of such intergradations makes it clear that the line of demarcation between amphiploids and autoploids is not very sharp, even though the cytogenetic requirements for success are sharply contrasted in the extreme cases.

Interplay between cytogenetic processes in polyploids. All the chromosomes in the autoploid have originated within one species, and each chromosome is therefore represented more than twice. No specifically new hereditary materials have been introduced through this chromosome addition, and, the chromosome homology being complete between sets in this newly created genome, the genic balance has not been disturbed and the autoploid can be essentially as fertile as its parent. The presence of more than one pair of each kind of chromosomes tends to obscure the effect of gene mutations and so is a stabilizing factor. On the other hand, the orderly distribution of more than two chromosomes of a kind during meiosis is frequently difficult.

In amphiploids the situation is much more complex, and the perpetuation of a successful balance between specifically distinct genomes is achieved in various ways. In general, the balance is attained through the interplay between two groups of antagonistic processes. One group tends to destroy the identity of duplicated genomes by (1) interchange of genes or chromosomes between parental genomes, and (2) chromosomal rearrangements. Either or both may take place either before or after doubling of the chromosomes. The other group tends to preserve the identity of the genomes by (1) preferential pairing (due to closer homology) between duplicate chromosomes of one parent, as compared with pairing between them and their nearest homologues from the other parent, and (2) elimination of unsuccessful combinations resulting from occasional interchange between unlike genomes. Interspecific sterility, both gametic and zygotic, and weakness of offspring are the most effective means of elimination. If the relationship of the parents permits, stability is obtained after these groups of processes have produced a new genome of successful character from which discordant rearrangements have been practically eliminated.

A classification of auto- and amphiploids is attempted in table 12, in which various influences that determine their success or failure have been coordinated and plotted one against another. Across the top are listed progressively the degrees of difference between the forms that give rise to auto- and amphiploids. These are given in terms of the biosystematic units. As a corollary, distinctions in genetic relationship are expressed through the degree of sterility in the undoubled F_1 generation. In the cases given in the central vertical column, in which the F_1 hybrid is partially sterile, the degree of sterility is often manifest through constitutional weakness of the undoubled F_2 generation. Also, it is to be noted that autoploids of nonhybrid origin are purposely omitted from this table.

In the two left-hand columns influences are considered that tend to preserve, versus those that tend to recombine, the parental genomes. Since the success of amphiploids largely depends upon the preservation of balanced genomes intact through succeeding generations, the mechanism that governs this process is given special consideration. As has been pointed out by Darlington (1929), the preservation of the initial balance is endangered if the chromosomes of the parents are so homologous that intergenomal pairing takes place. If such pairing is complete, one should expect in all cases to find the identity of the original genomes

TABLE 12

CIRCUMSTANCES OF ORIGIN OF HYBRID AUTOPLOIDS AND AMPHIPLOIDS

		AUTOPLOIDS	AMPHIPLOIDS	
		INTRA-ECOSPECIFIC: Undoubled F_1 fully fertile	INTER-ECOSPECIFIC: Undoubled F_1 partially sterile	INTER-CENOSPECIFIC: Undoubled F_1 wholly sterile
PARENTAL GENOMES:	INTERGENOMAL PAIRING:			
Identity lost in recombination	Complete	1. Fragaria bracteata × vesca rosea (also many nonhybrid cases)	2. Primula kewensis 3. Aquilegia Janczewskii	Probable
	Partial	....	4. Crepis rubra × foetida	5. Crepis capillaris × tectorum
	None	....	Improbable	6. Layia pentaglossa
Identity preserved	Partial	....	7. Galeopsis Tetrahit 8. Erophila duplex × quadruplex	9. Digitalis mertonensis
	None	....	Probable	10. Phleum pratense 11. Triticale 12. Raphanobrassica 13. Brassica Napus 14. Gossypium Thurberi × arboreum 15. Nicotiana Tabacum 16. Nicotiana digluta 17. Madia nutrammii 18. Madia citrigracilis

lost in recombination, so the alternative combination, complete intergenomal pairing with the identity of parental genomes preserved, is omitted from the table. But even if the parental chromosomes do not pair, the identity of the genomes may nevertheless be lost through the random distribution of unpaired chromosomes, or through the reciprocal translocation of segments of chromosomes. Also, the identity of the genomes may be preserved even after partial intergenomal pairing; for a strong gametic selection may eliminate unfit combinations, or the attraction between the chromosomes from one parent, after doubling, may outweigh the attraction of their nearest homologue from the other.

In table 12 genetic relationships of the parents are closest in the upper left-hand corner, and become progressively more remote down to the lower right-hand corner. In the various boxes well known examples are placed, to give our conception of this scheme of classification in operation. Each example is reviewed in later paragraphs. Boxes for which no examples are as yet known to us are marked as to the probability of finding cases for them. The combinations in the autoploid column below the first instance appear obviously incongruous. In general, it will be noted that the autoploids fall in the box in the upper left-hand corner, and the most stable amphiploids in the lower right-hand corner, following the order from closest to most remote relationship of the parents.

REVIEW OF EXAMPLES OF POLYPLOIDY

Eighteen well documented cases of polyploidy are listed by number and name in the boxes of table 12. In this classification special attention has been paid to amphiploidy. The only instance of autoploidy given is one that has been widely cited as an example of amphiploidy. A discussion of the many known cases of autoploidy falls somewhat outside the scope of this review, although in chapter VIII the ecological relations of some of the clearest examples of autoploidy are considered.

The various cases of amphiploidy have been classified after a careful examination of all the evidence. Relatively few examples are sufficiently well documented to permit their placement in this classification. This limitation has made it necessary for the authors to confine their attention to the instances that follow. A fuller list of amphiploids has been summarized by Goodspeed and Bradley (1942).

In order to evaluate the examples properly, it has been necessary to construct pedigrees giving the complete history of the amphiploid. These were obtained from all the available data, including (1) crossing trials, both successes and failures; (2) pollen fertility and seed setting in the parents as well as in both the undoubled and doubled hybrids; (3) germination, vigor of hybrid offspring, and segregation of characters in the undoubled F_2 and in the amphiploid; and (4) pairing of the chromosomes, especially in the undoubled F_1 and in the amphiploid. It was necessary, in addition, to construct a crossing diagram of the comparium to which the parents of the amphiploid belong in order to evaluate properly the biosystematic relations among the different members of the group. The findings of various investigators were superimposed in these diagrams in order to obtain as complete a picture as possible of the genetic situation in each comparium.

Facts pertaining to the eighteen examples are summarized below in the order in which the examples appear in the table. The documentary data, in small type, are followed by an interpretive discussion of the biosystematic principles involved. In this way, application of the principles is illustrated by case histories of some very well known groups of plants.

1. *Fragaria bracteata* Hell., $n = 7$, × *F. vesca* L. var. *rosea* Rostr., $n = 7$ (Ichijima, 1926, 1930; Yarnell, 1931*b*). One of the F_1 plants of this hybrid was tetraploid, with 14 pairs of chromosomes. This case has been widely cited as an instance of a fertile amphiploid arising from two species with homologous chromosomes, but this conclusion does not take into account the biosystematic facts.

This so-called amphiploid occurred in a cross made by A. J. Mangelsdorf at the Bussey Institution in 1924. Considerable confusion has arisen as to the names of the varieties employed in this cross, because in Ichijima's first publication the *Fragaria vesca* variety was called *F. Helleri* Holz., and in the 1930 paper this identification was corrected to *vesca* var. *rosea* without reference to the name under which the plant had appeared previously. *Fragaria Helleri* is an obscure, pink-flowered Idaho strawberry of doubtful taxonomic standing, but the source of Mangelsdorf's plants has not been given.

According to Mangelsdorf and East (1927) and Yarnell (1931*a*), the other parent, *F. bracteata*, belongs to a large complex of forms that includes *F. vesca*, *americana*, *mexicana*, *californica*, and a Hawaiian form. These intercross freely and produce fertile, vigorous hybrids, and all have 7 pairs of chromosomes which pair perfectly in the hybrids. This evidently means that these forms are but subspecies or ecotypes of one large ecospecies, *Fragaria vesca* L., which is circumpolar in its distribution.

The artificial tetraploid therefore arose from a hybrid not between distinct species, but between subspecies of one species. Consequently, it is not an amphiploid, as it is usually represented, but an autoploid.

Mangelsdorf obtained 10 pink-flowered F_1 plants of the cross *bracteata* (white) × *vesca rosea* (pink). Nine of these were diploid, with 7 pairs of chromosomes, and segregated normally in the F_2 (Ichijima, 1926). One F_1 plant was tetraploid, with 14 pairs of chromosomes. It probably originated through somatic doubling early in its development. A non-segregating, pink-flowered F_2 of this plant was grown, but it consisted of only 7 individuals, all with $2n = 28$ chromosomes. On the basis of this evidence some authors have listed this autoploid as constant, not realizing that its further history has been reported by Yarnell (1931*b*). The F_2 plants were selfed, and yielded 73 F_3 plants. Likewise, they were crossed with white-flowered, recessive, diploid forms of the *vesca* complex, from which some 400 offspring were obtained. Both selfs and crosses segregated pink- and white-flowered forms, proving that tetraploidy did not make this form constant, but that the chromosomes of *bracteata* and of *vesca rosea* were interchangeable, as would be expected in an autoploid. Even the chromosome number varied, for in addition to plants with the normal number, $2n = 28$, others appeared with 27, 29, 30, and 35 chromosomes. Similar aberrations were found in the outcrossings, with 28 plants having $2n = 22$, and 21 plants having $2n = 20$, in addition to the normal triploids with $2n = 21$.

All genetically investigated species of *Fragaria* belong to one comparium, in which *Duchesnea indica* (Andr.) Focke is also included. The polyploid series of chromosome numbers in *Fragaria* runs, $n = 7, 21$, and 28, with $n = 42$ known in *Duchesnea indica.* (The latter species is often included in *Fragaria.*) The synthesized autotetraploid described by Ichijima and Yarnell, with $n = 14$, is of interest because it fills a gap in this polyploid series.

Probably all the cytogenetically analyzed strawberries can be included in about five ecospecies, divided among two cenospecies. There are three known diploids, with $n = 7$. The principal one of these is *Fragaria vesca* L., which occupies an extensive region almost throughout Eurasia and in parts of North America. This species of woods and fairly moist habitats has developed a large number of climatic and altitudinal ecotypes. In North America several of these have received specific names, for instance, *F. americana* (Port.) Britt., *F. bracteata* Hell., *F. californica* Cham. et Schlecht., and *F. mexicana* Schlecht.

A second 7-chromosome ecospecies is *F. viridis* Duch. (= *F. collina* Ehrh.), a native of open, dry, warm habitats in Europe. It is only partially interfertile with the various races of *vesca,* whereas the latter are completely interfertile among themselves. Another 7-chromosome species is *F. nilgerrensis* Schlecht. ex J. Gay, of China and India, which crosses with the others with difficulty.

As has been mentioned, no wild 14-chromosome species is known, but there is a hexaploid, with $n = 21$, *F. moschata* Duch. (= *F. elatior*

Ehrh.). This native of temperate Europe belongs within the cenospecies containing the diploids, because it is partially interfertile with them.

The second known cenospecies contains the octoploid forms, $n = 28$, to which our cultivated strawberries belong. Both known octoploid groups are American. One is the western coastal strawberry, *Fragaria chiloensis* (L.) Duch., which occupies a narrow strip along the Pacific Ocean from Alaska to California, and in South America from Perú to southern Chile. Perhaps it has developed several latitudinal ecotypes. The other is *F. virginiana* Duch., which is common in temperate North America, where under many names it extends from the Atlantic coast to the Sierra Nevada. The investigations by Mangelsdorf and East (1927) and Yarnell (1931*a*) showed that forms of the two octoploids from the eastern United States, Canada, the Sierra Nevada, and the Oregon coast produced vigorous and fully fertile hybrids, but no information on the vigor of the F_2 was given. Hybrids between the octoploid and diploid species, and between the octoploid and hexaploid species are completely sterile, so no exchange of genes is possible between them.

From the facts available it is not possible to develop a clear picture of the evolution of the strawberries, possibly because too many ancestral species have already been exterminated. The newly synthesized tetraploid form, however, illustrates what happens when genome doubling takes place in a hybrid between even unlike races of the same species. A polyploid of this origin remains fertile and successful even though an exchange of parental chromosomes occurs, because the close relationship of the parents permits a free interchange of their genes without deleterious consequences. In such polyploids, therefore, the characters distinguishing the parents will segregate. The future of autoploids developed in nature depends upon whether or not they are adapted to fill the available ecological niches.

The following seventeen cases represent classes of amphiploidy.

2. *Primula kewensis*, $n = 18$; from *P. floribunda* Wall., $n = 9$, × *P. verticillata* Forsk., $n = 9$ (Newton and Pellew, 1929). The parents are native in Afghanistan and Arabia, respectively. The amphiploid originated through somatic doubling.

The original hybrid was diploid, $2n = 18$. It arose spontaneously at Kew in 1899 as a single individual, and later some hybrids were obtained artificially to confirm the parentage of the first. Subsequent attempts to repeat the cross failed. This diploid was described as × *P. kewensis* W. Wats. in 1900. It was vigorous, female sterile, but 1.5 per cent male fertile and able to exchange genes with its *floribunda* parent. Its chromosomes paired normally. Fertile shoots appeared on the diploid F_1 three times: in 1905, 1923, and 1926. Tetraploid F_2 seedling populations were raised from these, and it was established that the

1926 clone member was a sectorial chimera, and that the fertile shoot from which the seeds came was tetraploid. Tetraploid *kewensis* was morphologically similar to its diploid progenitor. From the 1923 and 1926 fertile shoots, 55 and 287 fertile F_2 individuals were raised. These were fairly uniform, although there was some segregation of the characters marking the specifically different parents. Quite a few plants with aberrant chromosome numbers ($2n = 34$, 35, and 37) appeared in later generations of the tetraploid. Such deviations were connected with weakness and sometimes sterility. This amphiploid has been observed at least through the F_4 generation.

Because there was a slight possibility for gene exchange through their diploid hybrid, the parental species should be considered remotely related ecospecies of one cenospecies. Newton and Pellew (1929, p. 460) expressed the view that the amphiploid *Primula kewensis* is not a good species, because the evidence points to an increased variability among the derivatives rather than a greater stability. This is to be expected from the following considerations: (1) the parents belong to one cenospecies and therefore are not very remotely related; (2) the chromosomes paired with little irregularity in F_1, so interspecific pairing would be anticipated in the amphiploid; (3) backcrosses of the diploid hybrid, as well as later generations of the amphiploid, showed some segregation of the original specific characters; and (4) the loss or gain of a chromosome or two in the amphiploid populations occasionally occurred. Upcott (1939) has thoroughly analyzed the pairing in diploid and tetraploid *P. kewensis* and its parents. This analysis suggests that the chromosomes of *P. floribunda* and *P. verticillata* are not completely homologous, although the irregularities are slight. *Primula kewensis* has received wide attention because it was the first amphiploid form to be investigated. Since it originated by somatic doubling and could be continued indefinitely through vegetative propagation, it appeared to be constant until the gametic tests disclosed its lack of stability.

3. *Aquilegia Janczewskii* Skal., $n = 14$; from *A. chrysantha* A. Gray, $n = 7$, × *A. flabellata* Sieb. et Zucch., $n = 7$ (Skalińska, 1935, 1937). The parents are native in the southwestern United States and Japan, respectively.

This cross produced 97 F_1 plants, of which 95 were diploid, 1 was tetraploid, and 1 triploid. The reciprocal cross produced 72 plants, all of which were diploid. The diploid F_1 plants ($2n = 14$) were vigorous and quite fertile, and their chromosomes paired regularly in meiosis. Nevertheless, there was considerable pollen sterility (the *flabellata* × *chrysantha* combination being almost male sterile), seed germination was not very good, and the diploid F_2 showed reduced vigor. A vivid description of the degeneration of another F_2 of the same combination was given earlier (Skalińska, 1929, p. 146).

Because the single tetraploid plant appeared in the F_1, it was supposed to have arisen through somatic doubling, but the presence of the triploid in the same progeny seems to weaken this argument. It was slightly irregular in its chromosome pairing (tetra-, tri-, and univalents occurred), and it seemed to be less fertile than the diploids, but it produced 168 F_2 plants, which showed no decrease in vigor. The tetraploid F_2 population segregated, but to a much lesser degree than the diploid F_2, and the pollen fertility averaged 25 to 50 per cent, rarely reaching 75 per cent. Seed production indicated that most of the sterility was due to bad pollen. This tetraploid population, having "doubtless the value of a new species," is called *A. Janczewskii.*

The triploid F_1 plant yielded an F_2 of 5 diploids and hyperdiploids and 8 tetraploids. These tetraploids were unlike those of the primary tetraploid, so that morphologically they would be assigned to another section of the genus. The two tetraploid groups, however, proved to be interfertile; but they showed a high degree of sexual isolation from the diploids of these and other species.

The partial sterility of the diploid hybrids between *Aquilegia chrysantha* and *flabellata* and the weakness of their second generation, combined with the considerable gene interchange of the species, indicate that these are fairly closely related ecospecies of one cenospecies. Because its parents were somewhat interfertile and some possibility existed for gene interchange between their chromosomes, it was to be expected that the amphiploid derivative would be somewhat sterile. The future of an amphiploid of this kind is uncertain, although biologically it represents an improvement over the less successful diploid hybrid.

The genus *Aquilegia* is exceptional in combining extreme morphological and ecological variability with a high degree of crossability. In other words, differentiation in this genus has proceeded a great distance morphologically and ecologically without developing distinct cenospecies, and the barriers that separate the units are more strongly geographic than cytogenetic. The genus encircles the northern hemisphere and has developed distinguishable forms that inhabit climates ranging from warm-temperate to arctic-alpine. During this dispersion and differentiation the genus failed to develop internal barriers to interbreeding, and all the wild forms remained on the diploid level with 7 pairs of chromosomes.

Payson (1918) divided the genus into three sections, but admitted that interspecific hybrids might be expected wherever two species are found growing together, even though the species belong to different sections. The present writers are well acquainted with swarms of evidently completely fertile intersectional hybrids in the high Sierra Nevada, where *A. formosa* Fisch. meets the high alpine *A. pubescens* Cov. All the sectional characters recombine in the individuals of these local populations, suggesting in this case the absence of even interspecific barriers.

Winge's investigations (1928, pp. 70, 319), in which several thousand F_2 individuals of inter- and intrasectional hybrids were analyzed, failed to disclose any strong barriers to interbreeding in the genus, and Skalińska's studies bear out this observation. Skalińska's amphiploid, which can scarcely be crossed back with any of several diploids, represents approximately the highest degree of intersterility known in the genus. It is an intersectional hybrid combining an American and an Asiatic form.

These results lead to the conclusion that *Aquilegia* is one huge cenospecies composed of only a few ecospecies. Probably most of the recognized "species" are merely morphologically distinguishable ecotypes or subspecies. This evolutionary status is of much interest, because it possibly represents a youthful stage experienced by many other, now mature genera, before they developed strong barriers to interbreeding, with polyploidy following. This is probably the most flexible stage in the evolution of a genus. It is now easier to see why *Aquilegia* has never succeeded in evolving polyploidy. It simply has not yet arrived at the proper evolutionary level for this pattern to develop successfully.

4. *Crepis rubra* L., $n = 5$, × *C. foetida* L., $n = 5$. The amphiploid from this cross had 10 pairs of chromosomes, with slight oscillations, but was weak and highly sterile (Poole, 1931, 1932). It originated through gametic doubling in the F_1.

Approximately 30 F_1 plants were produced in three crosses by Haney, Navashin, and Poole (Babcock and Navashin, 1930, p. 80; Poole, 1931, pp. 174, 183). Little was learned of chromosome pairing in the F_1 except that "instances of complete pairing" occurred despite morphological differences between some pairs. *Crepis* chromosomes are few and large, and often of recognizable morphology. Two pairs of parental chromosomes in this cross were sufficiently distinct so that their distribution could be traced through later generations.

The F_1 plants were slightly fertile in backcrosses with each parent. In the F_2 of backcrosses to *rubra,* 100 plants germinated but only 30 survived. In these, extensive segregations were observed. For example, although this backcross was made to *rubra,* certain plants transmitted 10, 13, and even 14 characters of *foetida.* The fertility improved in the second generation of the diploid backcrosses.

An F_2 generation was raised from a single selfed F_1 plant. This F_1 was 2.7 per cent fertile and produced 9 F_2 plants, 3 of which were diploid, 5 triploid, and 1 tetraploid. The 20 chromosomes of the single amphiploid varied in their pairing between the extremes of 10 pairs and 5 quadrivalents. From selfing, this plant produced an F_3 of 45 plants. Of these, 23 were tetraploid, and the other 22 had between $2n = 19$ and 22. Thirteen of the tetraploids showed visible rearrangements in their chromosomes, indicating that translocations between parental sets interfered with the preservation of their identity. What interchanges had taken place between the morphologically similar chromosomes could only be surmised through the segregation of specific characters. Fertilities of the F_3 varied from 0 to 9 per cent. F_4 populations were grown from three of the most fertile F_3 plants, with 20, 20, and 21 chromosomes, respectively, but now the fertilities decreased to 0.6 per cent. All F_4 plants varied in chromosome number between $2n = 19$ and 22, with a distinct node at 20. They continued to show extreme diversity and visible reorganization of their chromosomes, but their high sterility prevented a continuation of this line.

The unusually complete investigation of this amphiploid shows clearly that simple doubling of the chromosome complement does not insure the success of a poor hybrid. The rather fertile backcrosses of the F_1 to *rubra* indicate that the parents are members of the same cenospecies. The few data on the behavior of the chromosomes in F_1 make our classification of the hybrid as having partial pairing rather arbitrary. Two correlated observations on this amphiploid are of special interest, viz., the striking recombination of parental characters, and the visible repatterning of the chromosomes. The fate of this amphiploid was sealed through too much interchange between the genomes of the parent species, followed by a progressive sterility through succeeding generations.

5. *Crepis capillaris* (L.) Wallr., $n = 3$, × *C. tectorum* L., $n = 4$ (Hollingshead, 1930*a*, 1930*b*).

Forty F_1 plants were obtained, half of which died in the cotyledon stage. Of the 20 plants which matured, 2 were fairly fertile, with 15 and 21 per cent good pollen, but the others were highly sterile, usually with less than 1 per cent good pollen. The most fertile F_1 plant was found to be triploid, with two sets of *capillaris* and one of *tectorum* chromosomes, but the other fertile plant was not examined cytologically. The sterile F_1 plants were diploid.

The chromosomes of *capillaris* and *tectorum* are partially homologous, for in the diploid hybrids 3 pairs plus 1 single were frequently observed, although all 7 chromosomes sometimes remained unpaired. Occasional dyads were seen among the tetrads. In the triploid F_1, 3 pairs plus 4 singles were usual, although it could not be ascertained whether the pairs always consisted exclusively of *capillaris* chromosomes, for the A chromosomes of the parents are morphologically similar.

The triploid F_1 plant produced 101 F_2 offspring with chromosome numbers ranging all the way from $2n = 6$ (59 plants, thought to be pure *capillaris*) to $2n = 14$ (1 supposed amphiploid). Less than half the pollen mother cells of the latter, however, had 7 pairs of chromosomes, the remainder varying from 6 to 4 pairs. The amphiploid differed from the parental species and the F_1 in not producing a rosette before flowering, but in other respects it resembled the diploid F_1. It had up to 32 per cent good pollen, but was characterized as weak, and no offspring of it were grown.

This amphiploid must be characterized as unsuccessful. It arose from the selfing of the triploid, presumably by the addition of gametes each containing full sets of *capillaris* and *tectorum* chromosomes. The partial homology between the parental chromosomes, however, could allow interspecific crossing over to take place before the doubling, in which case the 14-chromosome plant might have been a quasi amphiploid, like *Layia pentaglossa*. Its rapid development and lack of a rosette are suggestive of some such change.

Crepis capillaris and *C. tectorum* are classified by Babcock (in press) in sections 24 and 14, respectively, indicating a distant relationship. This conclusion is supported by the genetic evidence that their genomes do not combine well. Hollingshead produced more than 2000 F_1 hybrids of various races of these two species. In some combinations, all the plants died in the cotyledon stage; in others, all survived; and in still others, half were inviable. She showed that this was caused by a single *tectorum* gene, which was detrimental only in hybrid combinations. Even without this complication, however, the viable F_1 plants are highly sterile, a fact which indicates that the parents probably belong to distinct cenospecies. When the parental sets of chromosomes are at the same time highly incompatible and partly homologous—an unusual situation—very little success can be expected from the resulting amphiploid.

The two recorded experimental amphiploids of *Crepis* were unsuccessful. It has been suggested, however, that the American *Crepis* (which, unlike the Old World species, have chromosome numbers in multiples of 11) originated from 4- and 7-chromosome Asiatic species through amphiploidy (Babcock and Stebbins, 1938).

6. *Layia pentaglossa*, $n = 15$; from *L. pentachaeta* A. Gray, $n = 8$, × *L. platyglossa* (F. et M.) A. Gray, $n = 7$. The history of this amphiploid is shown in the pedigree, figure 48, page 49.

To summarize briefly, only 22 moderately vigorous F_1 hybrids were obtained, and none in the reciprocal cross. Doubling was gametic through diploid sex cells of the F_1; such cells were observed. There was very little pairing in the F_1, and its effective fertility was only 0.12 per cent. Only 16 F_2 plants were obtained from 8 F_1's open-pollinated throughout the season in an isolation plot. Two of these were diploid or near diploid, 11 were triploid, and 3 were tetraploid or near tetraploid. The segregation observed in these indicated that extensive chromosomal exchanges had taken place between the parental genomes (table 10, p. 51). This F_2 was highly sterile but produced a relatively uniform F_3 of 11 amphiploid plants ($2n = 30$) and one 29-chromosome plant. The amphiploids had 14 to 15 pairs of chromosomes and 0 to 2 singles, but were entirely sterile. The 8 best plants were open-pollinated in an isolation plot, but no offspring were obtained.

The parents of *Layia pentaglossa* belong to distinct cenospecies of one comparium. This comparium is almost synonymous with the genus. As in *Crepis rubra* × *foetida*, the lack of success of this amphiploid is accompanied by extensive recombination of the parental genomes. The *Layia* differed from the *Crepis* in that the exchange took place before the chromosomes doubled, but this did not alter the results. Because the chromosomes of the parent species are morphologically fairly similar, it was impossible fully to trace their identities in the hybrids.

7. *Galeopsis Tetrahit* L., $n = 16$; from *G. pubescens* Bess., $n = 8$, × *G. speciosa* Mill., $n = 8$ (Müntzing, 1930*a*, 1930*b*, 1932).

The parental species produced a quite highly sterile F_1 population of 7 plants (pollen fertility 8.25 per cent). Five to 8 pairs of chromosomes were found, however, showing that there was a partial homology between the parental genomes. A diploid F_2 generation of 197 individuals was raised, which segregated extensively and had an average pollen fertility of 22 per cent. One F_2 plant was found to be triploid and almost completely sterile. It had probably arisen from one unreduced and one reduced gamete of the F_1 hybrid. Backcrosses to *pubescens* yielded a single seed on the triploid, which gave rise to a tetraploid plant. This must have arisen through the union of an unreduced triploid ovule and a haploid sperm. Occasional fusions of the nuclear spindles in second metaphases of the F_1 and the triploid F_2 plant suggested a mechanism for such a union of all the chromosomes. The tetraploid F_3 plant had 16 pairs of chromosomes in first metaphase and was 70 per cent male fertile. Both the triploid F_2 and its tetraploid derivative were indistinguishable from *G. Tetrahit* but quite different from the F_1.

An F_4 generation grown from the selfed tetraploid F_3 plant consisted of about 190 plants. These were variable, but two-thirds of them were morphologically indistinguishable from *G. Tetrahit*. They differed individually as to fertility, but averaged 68 per cent male fertile. In a sample of 33 plants, 24 had the normal chromosome number ($2n = 32$), 4 were probably normal, and 5 had $2n = 33$ chromosomes. Like the wild *Tetrahit*, this culture was unable to cross with its diploid parents, but it crossed easily with nine different strains of wild *Tetrahit*. The offspring of these crosses were 70–82 per cent male fertile in five combinations, and 47–52 per cent in the other four, in this regard being similar to the wild strains of *Tetrahit*.

These three species of the mint family are morphologically closely related, the two diploids having at times been considered to be subspecies of the tetraploid *G. Tetrahit*. Both *pubescens* and *speciosa* occur in central and eastern Europe. The partial barriers to interbreeding indicate that they are distinct ecospecies of one cenospecies. *Galeopsis pubescens* tends to be a lowland and foothill ecospecies, whereas *speciosa* is more montane or even subalpine, extending to latitude 70° north on the Scandinavian peninsula. The tetraploid descendant, *Tetrahit*, has a much wider distribution than either of the supposed parents, extending from the Faeroe Islands to Siberia, Japan, and northwestern India. It extends as far north as the more montane of the diploid species, and considerably higher in the mountains. Accordingly, it has been very aggressive, and the artificial synthesis of a successful form of it becomes of special interest.

Synthetic *G. Tetrahit* did not arise as a straight doubling of the F_1 hybrid (*pubescens* × *speciosa*), but in two steps via a triploid. The supposedly haploid F_1 gamete that entered into this triploid probably contained a partially recombined genome, for the synthetic *Tetrahit* is morphologically different from the F_1, and in this respect unlike other amphiploids. Natural *Tetrahit* is widely variable, but none of its forms are like the F_1 of *pubescens* × *speciosa*. Nevertheless, it may possibly have arisen several times in the wild from F_2 products of this cross. Natural hybrids between these species are known to occur.

8. *Erophila duplex* Winge, $n = 15$, × *E. quadruplex* Winge, $n = 32$. A constant and fertile amphiploid ($n = 47$) arose from this hybrid in F_2 as a result of the union of diploid gametes (Winge, 1933, 1940).

The 15-chromosome parent, from Denmark, and the 32-chromosome parent, from Holland, are constant and readily distinguishable. Their F_1 offspring, consisting of 14 plants, were quite sterile. The cytological analysis is incomplete, but dyads and diploid pollen grains with 47 chromosomes were observed, in addition to tetrads with cells of unequal size. A total of 115 F_2 plants were grown and showed "no conspicuous segregation." Ten F_2 plants were studied cytologically as a sample; at least 4 of them had $n = 46$–48 chromosomes, and they with two others had regular pollen, were fertile, and produced constant F_3 offspring. The other 4 plants had irregular meiosis and pollen, and one of them segregated strongly in F_3. Some selfed lines became constant in F_3, others in F_4. This amphiploid was continued through the F_5, and was stated to be more vigorous than any natural type (in keeping with its having the highest known chromosome number, because plant size increases in proportion to chromosome number in this genus), but it had only one or a few peduncles and flowered late.

The genus *Erophila* is the old Linnaean species *Draba verna.* This group of diminutive spring-flowering annuals is a very complex cenospecies with chromosome numbers ranging between $n = 7$ and $n = 32$. They form a dysploid series, but there are conspicuous interruptions in it, for no forms have been found with the numbers 8 to 11, 13, 14, and 21 to 25. Forms with adjacent chromosome numbers are highly interfertile, but more or less intersterile with other groups. These interbreeding chromosomal races have characteristic geographical and ecological distributions. Winge found that four such groups could be recognized, and he proposed to call them species, as follows: *E. simplex*, $n = 7$; *E. semiduplex*, $n = 12$; *E. duplex*, $n = 15$–20; and *E. quadruplex*, $n = 26$–32. Biologically, these are ecospecies of one cenospecies. These Erophilas, therefore, have a very flexible system of chromosome balances, since in the higher brackets of the chromosomal series, forms with adjacent chromosome numbers are intercompatible in breeding. The new amphiploid represents a new chromosomal balance, the highest in the genus.

Erophila verna (L.) E. Mey. has been subjected to many intensive experimental investigations since 1850, when Alexis Jordan started to cultivate it and found that the complex consisted of many true-breeding, distinguishable forms. Winge's experiments have shown that even different ecospecies can be found growing side by side in one population, because the plants are self-fertile and cross only occasionally. Also, his creation of a new constant type of different chromosome number demonstrates that new types may thus arise in nature. These facts point to further differentiation in the genus, and make it evident that the cenospecies is in a very active stage of evolution.

The multitude of forms and the minute size of the plants have made taxonomic studies of this group hitherto exceedingly unsatisfactory. Winge's demonstration, by the application of biosystematic principles, that despite the large dysploid series of chromosome numbers the complex is actually organized into four fairly distinct ecospecies is a most valuable contribution to *Erophila* taxonomy.

9. *Digitalis mertonensis*, $n = 56$; from *D. purpurea* L., $n = 28$, × *D. ambigua* Murr., $n = 28$ (Buxton and Newton, 1928; Buxton and Darlington, 1931). The parental species are native to Europe. The amphiploid arose in the F_2 generation presumably by the addition of unreduced gametes known to occur in the F_1 hybrid.

Fifty F_1 plants were obtained with *D. purpurea* as the female parent, but none from the reciprocal cross. These had $2n = 56$ chromosomes. They were vigorous, morphologically intermediate between the parents, and very sterile. In all, 43 F_1 plants flowered, but only 2 showed some degree of fertility, although a few others set stray seeds. The chromosomal pairing was poor, for only 5 to 12 pairs were seen among the 56 chromosomes. It could not be determined whether this was intra- or interspecific pairing. Meiosis was usually very irregular, but frequently restitution nuclei were formed, containing all the chromosomes from both parents. Some dyads were also observed among the tetrads.

About 200 F_2 plants were grown; 110 of these originated from controlled pollination, of which 96 flowered. There was no apparent segregation in this generation, for it duplicated the F_1 except that the flowers were distinctly larger. Of 58 plants which flowered the second year after sowing, about 75 per cent "set some well filled capsules," and the remainder were essentially sterile. Nine of the 10 fertile plants whose chromosome number was determined had $2n = 111–112$, and 1 had $2n = 102$. In the true amphiploids the meiotic divisions were generally regular, though occasional univalents were seen in all plants, and the bivalents sometimes aggregated into groups of 2, 3, and 4. This amphiploid was grown through the F_5 generation. It remained tetraploid and giant, but in each generation approximately one-fourth of the plants were less fertile than the others.

Of the 90 seedlings raised from open-pollinated F_1 plants, 72 reached the flowering stage. Again there was no evident segregation, and all were presumably triploids, for the 8 plants counted had 84 chromosomes without exception. They must have originated from diploid ovules of the hybrid backcrossed to the parental species. Only from 25 to 30 per cent of these plants set a little seed.

Digitalis purpurea and *D. ambigua* behaved as members of distinct cenospecies of one comparium, for there was no exchange of chromosomes between them in the hybrid. Although the F_1 yielded some 200 F_2 plants, these were either amphiploids or nearly sterile triploids. All the fertility resulted from the addition of the chromosome sets of both parents in the only functional hybrid gametes. The new amphiploid, *Digitalis mertonensis*, does not freely exchange genes with the parental species. In crosses with them only highly sterile triploids are formed, so there is no difficulty in preserving the artificial new species uncontaminated by crossing.

10. *Phleum pratense* L., $n = 21$; from *P. nodosum* L., $n = 7$, × *P.* alpinum L., n = 14 (Gregor and Sansome, 1930; Gregor, 1931; Nordenskiöld, 1937).

This cross was easily made in both directions by Nordenskiöld, but Gregor did not produce the reciprocal combination. Gregor studied about 600 F_1 plants. These were more like the *alpinum* parent in appearance, very sterile, and triploid ($2n = 21$). No offspring were obtained from controlled greenhouse pollinations, and the entire yield from some 500,000 open-pollinated flowers in the field was 46 seeds. Only 4 F_2 plants were produced from them. The 3 of these that were examined cytologically were hexaploid ($2n = 42$), and must have arisen from ovules containing complete genomes of *nodosum* and *alpinum*, pollinated by 21-chromosome pollen of the F_1, or by *pratense* from the fields. Three of the 4 F_2 plants were either male or female sterile or both, but the fourth was quite fertile. When this artificial hexaploid was crossed with the natural *pratense*, 161 hybrids resulted, many of which were both male and female fertile.

This series of experiments indicates that ovules of *pratense* were synthesized by the addition of genomes of *nodosum* and *alpinum*, and if the artificial hexaploid was produced by F_1 pollen, the full synthesis of *pratense* was achieved. The new product had at least a moderate degree of cross-compatibility with natural *pratense*.

Gregor and Sansome did not investigate meiosis, and apart from the fact that hexaploid F_2 plants were produced, there is very little indication of what kinds of gametes this hybrid had. In a backcross to *alpinum*, with the F_1 hybrid as the female parent, 5 totally sterile plants were obtained. In 3 of these, Sansome found $2n = 26$, 27, and 30. Because *alpinum* pollen has 14 chromosomes, the functional hybrid ovules in this case must have had 12, 13, and 16 chromosomes, respectively. This is considerably fewer than in the 21-chromosome ovules which produced the synthetic *pratense*.

Nordenskiöld reported the chromosomal pairing in various hybrids of the *Phleum pratense* group. She found that the *nodosum* × *alpinum* F_1 hybrid had up to 7 pairs plus univalents and occasional trivalents and quadrivalents. From the pairing in this single combination it is impossible to decide whether the 7 pairs were composed of the 14 *alpinum* chromosomes pairing inter se, or of the 7 *nodosum* chromosomes pairing with 7 from *alpinum*. The very high sterility makes it rather unlikely that all three sets of 7 are essentially homologous.

If the set of 7 chromosomes in the gametes of *nodosum* is represented by N, and the two sets of 7 in the gametes of *alpinum* by A_1A_2, the formula of the synthetic *pratense* gametes should be written NA_1A_2. A crucial test for the homologies of these sets is found in the pairing of *pratense*, $n = 21$, × *nodosum*, $n = 7$. The somatic formula for this hybrid would be NNA_1A_2. If the two sets of A chromosomes are homologous, this hybrid should have 14 pairs of chromosomes, instead of the 7 pairs plus 14 singles normally expected in a hybrid between a 21- and a 7-chromosome species. Nordenskiöld found 14 pairs. Both male and female fertility of this hybrid were good, and it is therefore likely that the sex cells of *alpinum* carry two essentially homologous sets of chromosomes, and that the *nodosum* set is not homologous with them. (The parental sets are not considered homologous in our table 12.) An occasional extra pair and multivalent association in the *nodosum* × *alpinum* hybrid probably indicate that two or three chromosomes of the parents are slightly homologous. Similar feeble pairing may be observed in even the remotest hybrids. Nordenskiöld uncovered a most unusual situation in the pairing and fertility relations in the chain of *Phleum* hybrids, but the behavior appears to find a natural explanation in the homology relations here suggested.

Considering their intersterility, *Phleum nodosum* and *P. alpinum* would appear to be members of distinct cenospecies. Thus far experiments have not shown that there is a chance for the direct exchange of genes between them. The evolution of *pratense* through the addition of their chromosomes, however, furnishes an avenue for an indirect exchange, so that today all three are members of one cenospecies.[1]

Morphologically, these three *Phleum* species are very closely related. For this reason *pratense* and *nodosum* have been so confused in the literature that it appears to be impossible to determine even whether *pratense* occurs as a wild species. The distribution of *nodosum* is fairly well known only in Scandinavia and Scotland.

The ecologic characteristics of the timothys are as distinct as the genetic. *Phleum alpinum* is the best separated, for it occurs at high elevations or at high latitudes in moist situations. Its distribution is circumpolar. *Phleum nodosum,* of dry, uncultivated areas, and *P. pratense,* of moister situations, are lowland species (Gregor, 1931). The most widely cultivated, *pratense,* is exceedingly adaptable, probably because it combines the inheritance of two species so unlike in their ecological requirements.

The origin of *pratense* is unknown. Probably it dates back to early historical times in northern Europe. Timothy was introduced from England into the eastern United States in 1720 by one Timothy Hansen. The improved tall hay type, returned to England in 1765, was supposed to have developed in America, but prior to this time it had been cultivated in Sweden. As early as 1759 Linnaeus clearly described the three species. His delimitation of them coincides with the chromosomal situation, and it is evident that he was familiar with the tall hay type in Sweden.

11. *Triticale*, $n = \pm 28$; from *Triticum vulgare* L., $n = 21$, × *Secale cereale* L., $n = 7$. This amphiploid has arisen independently several times, but the mode of origin has been most thoroughly demonstrated by Münt-

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[1] The diploid so-called "*alpinum*" of Nordenskiöld and Gregor produced partially fertile hybrids with *P. nodosum* but entirely sterile hybrids with true tetraploid *alpinum,* from which it is somewhat distinguishable morphologically. It is therefore a distinct ecospecies related to *nodosum* and probably not a diploid progenitor of true *alpinum.* Its native habitat is unknown, but the experimental material of these authors came by way of Svalöf from a south German experiment station, the only known source of this diploid. True *alpinum* has been found to be tetraploid wherever it has been investigated, namely, in northern Sweden (the type locality), Scotland, the Alps, and the Sierra Nevada of California.

zing, who summarizes the other instances (1936*a*, 1939). In order of origin the various strains are as follows:

(1) *The Rimpau strain* (German). Developed by W. Rimpau about 1890. Open pollination of the hybrids was permitted, but a strain was developed which was fairly constant, intermediate, and fertile. The true nature of this strain was not realized before 1935, when Lindschau and Oehler announced its chromosome number as $2n = 56$—a number verified by Müntzing (1936*a*).

(2) *The Meister strain* (Russian). Developed by a group of investigators at the Saratov Experiment Station, Ukraine, headed by G. K. Meister (1928). The original wheat-rye hybrids were open-pollinated. When the F_1 was backcrossed to the parents, three forms segregated: wheatlike, intermediates, and ryelike. Some intermediates were highly sterile, but others had nearly normal fertility and were amphiploid, with $2n = 56$. A cytological study was made by Levitsky and Benetzkaia (1931) on 13 plants of the F_4, F_5, and F_6 generations of families descended from three selected F_2 plants. The *Triticale* strains of this origin were constant, intermediate, and fertile, with the somatic chromosome number exactly 56 in all 13 plants examined. Since considerable chromosomal irregularity was observed at meiosis (up to 50 per cent of the pollen had more than the normal 28 chromosomes), it was assumed that only those gametes with 28 chromosomes were functional.

(3) *The Lebedeff strain* (Russian). Described by Lebedeff (1934). The F_1 plants of wheat × rye were intermediate in appearance and very sterile (no anthesis of anthers, and pollen not more than 0.12 per cent fertile). When the F_1 was backcrossed to rye, the F_2 plants more resembled rye, except one plant which resembled the F_1 parent and had $2n = 56$ chromosomes. Another case arose from an earlier wheat × rye cross. These F_1 plants were only 0.08 per cent fertile, except a group from one wheat plant that averaged 3.7 per cent. Thirteen of the F_2 plants produced from these relatively fertile hybrids were cytologically examined: 11 had $2n = 56$ chromosomes, 1 had $2n = 53$, and 1 had $2n = 30$. Lebedeff attributed the development of the amphiploids to apomixis, not considering the fertility of 3.7 per cent sufficient to account for self-pollination.

(4) *The Taylor strain* (American). Described by Taylor and Quisenberry (1935). In a series of experiments designed to test the inheritance of crossability of wheat with rye, a strain similar to wheat was produced that was crossable with rye. Of the 20 highly sterile F_1 hybrids of wheat-rye-wheat × Abruzzes rye obtained, 2 dehisced some of their anthers and 1 set a single grain. From this a nonsegregating line originated, intermediate between wheat and rye. Müntzing (1939) investigated this strain and found it had $2n = 56$. The devious procedure of double backcrossing did not prevent this amphiploid from appearing.

(5) *The Svalöf strain* (Swedish). Developed by Müntzing (1936*a*, 1939). Sixty-one F_1 plants were obtained from fifteen different combinations of wheat × rye, and all had $2n = 28$. Only 5 of these survived the winter, and only 2 had a little viable pollen. The pollen was of all sizes, but the plump grains were unusually large and were presumed to be diploid or an even higher multiple. Because the fertile anthers were distributed over the plants, a chimeral structure was not suspected. The four spikes with pollen were bagged, but only one seed developed. This supplied the only F_2 plant, which was approximately octoploid. For these reasons this F_2 is supposed to have arisen from the union of unreduced gametes following self-pollination.

The behavior of wheat and rye chromosomes during meiosis has been investigated by a large number of authors, including Kihara, Nikolaeva, Thompson, Zalensky and Doroshenko, Kagawa and Chizaki, Aase, Bleier, Longley and Sando, Liljefors, Florell, Lebedeff, Kattermann, and Plotnikova. The hybrids they investigated were produced from very different strains of wheat and rye, yet all authors agree that there is very little pairing, and that the few pairs observed (usually 0–3) are long and drawn out like those in the *Madia* and *Layia* hybrids described above. Consequently, there is practically no homology between wheat and rye chromosomes. Good illustrations of the meiotic behavior are found in the papers of Kihara (1924) and Aase (1930). Kihara observed dyads (1924, fig. 68) and reported that the extra large pollen grains were usually able to germinate, but no amphiploids of this combination are reported from his cultures.

The *Triticale* strains all apparently originated in the same striking manner, when the circumstances were highly favorable. The Svalöf strain, with very little doubt, originated from unreduced gametes, and the Lebedeff and Taylor strains must have arisen similarly. The modes of origin of the other two strains are unknown.

Irregularity in meiosis occurs to some degree in all *Triticale* strains, these differing by averages of from 2 to 8 univalents. According to Müntzing (1939), pollen grains with about 28 chromosomes survive, and also ovules with 26, 30, and 31 chromosomes in addition to those with the normal 28. But in backcrossing to wheat, plants with about 56 somatic chromosomes survived better and were taller and more fertile than those with deviating numbers. Therefore, the genic balance relations afford an effective mechanism for maintaining a fairly constant chromosome number despite distributional irregularities. The operation of this selective mechanism is well illustrated by the Rimpau strain, which, after some fifty generations, still approximates 56 chromosomes in spite of having an average of 6 unpaired chromosomes at meiosis. Wild species of plants are occasionally found with chromosome numbers oscillating within narrow limits without obvious ill effects. This is more common in the higher polyploid brackets (J. Clausen, 1931*a*).

Wheat and rye evidently belong to almost distinct cenospecies of one comparium. In the classification in table 12 they are regarded as belonging to different cenospecies. The F_1 between them is highly sterile except for the rare amphiploid offspring, which represents a new species. The F_1 is slightly interfertile with the wheat parent, however, and sometimes also with the rye. In backcrossed offspring, Kattermann (1938) developed a 21-paired form in which one pair of readily identifiable rye chromosomes substituted for a wheat pair. This experiment shows that there are slight possibilities for gene exchange between wheat and rye, although the two are quite effectively separated from each other. Biologically, therefore, one should not consider that wheat and rye belong to distinct genera.

The economic implications of *Triticale* are as interesting as the biological, since this amphiploid contains the genomes of the world's two chief sources of bread. Biologically, it is significant that *Triticale* has arisen at least five times under different conditions and from very different stock. These strains are only partially intercompatible, for Müntzing has shown that their hybrids are partly sterile. From these considerations it follows that if *Triticale* were exposed to natural conditions, it could undergo a process of selection and evolution that would possibly lead to several new species on the octoploid level.

OTHER AMPHIPLOIDS OF THE TRITICUM COMPARIUM. *Triticale* exemplifies the amphiploids of the *Triticum* comparium, a considerable number of which are on record. Another is of special interest because of its morphological and genetical relationship to common wheat. This 21-chromosome amphiploid was synthesized from *Triticum turgidum* Steud., $n = 14$, × *Aegilops speltoides* Tausch, $n = 7$ (Thompson, Britten, and Harding, 1943).

The first generation of this hybrid contained $2n = 21$ chromosomes and was very highly sterile. The chromosomes of *A. speltoides* are partially homologous with one of the sets of *T. turgidum*, for Jenkins (1929) usually found 7 loose pairs plus 7 singles in meiosis of this hybrid. Thompson and his coworkers found 4 or 5 pairs to be most frequent, and pointed out that there were trivalents in 80 per cent of the cells.

When seedling F_1 hybrids were treated with injections of 0.2 per cent colchicine, two plants produced sectors with quite fertile spikes. Seedlings from these were mostly amphiploid, with $2n = 42$ chromosomes, but several were also found with $2n = 40$ and 41. Pairing in the 42-chromosome plants was fairly regular, but usually about 3 or 4 univalents, a trivalent or two, and sometimes a tetravalent were observed. The amphiploids resembled the F_1 in most characters, and the amount of variation in genetic characters was "surprisingly small in view of the meiotic irregularities." They were grown through the F_3 and F_4 generations, in which a few plants continued to show deviations from the true amphiploid chromosome number. The fertility of the amphiploid was reasonably high, although much lower than that of the cultivated wheats, for only 37 per cent of the F_3 and F_4 plants had 10 or more grains per spike.

In a number of morphological characters the *Triticum turgidum* × *Aegilops speltoides* amphiploid resembled the common bread wheat, *T. vulgare* Vill., $n = 21$, but in others it resembled the emmer wheats, $n = 14$, and in a few, *T. Spelta* L., $n = 21$. It was crossed with both *vulgare* and *Spelta* and set seed well in both cases. These seeds, however, were shriveled and germinated poorly, giving only 8 F_1 plants. The hybrids with spelt averaged 4 seeds per spike, and those with *vulgare*, 7.

The *T. turgidum* × *A. speltoides* amphiploid should probably be classified with *Digitalis mertonensis*, for its undoubled F_1 is sterile, the chromosomes of its parents are partially homologous, and there appears to be very little exchange between the parental genomes in the amphiploid. Evidently it is not conspecific with either *T. vulgare* or *T. Spelta,* but is a new member of their cenospecies. In order to reproduce *T. vulgare,* it would probably be necessary to use as one parent a diploid *Aegilops* with chromosomes homologous with one set of *vulgare,* but not with either set of the 14-chromosome emmer wheats, and having the proper morphological characteristics. The partial homology between the chromosomes of *Aegilops speltoides* and some of the chromosomes of *Triticum* is a strong indication of a common evolutionary origin, emphasizing the fact that biologically *Aegilops* and *Triticum* belong to one genus.

The most extensive series of amphiploids on record from one group of experiments was synthesized by Sears (1941*a*, 1941*b*). By the colchicine method he produced eighteen amphiploids from the hybrids of eleven 7-paired members of the *Triticum* comparium. With one exception, all the undoubled F_1 hybrids giving rise to these amphiploids were essentially sterile. He made detailed studies of the pairing in the undoubled F_1 hybrids and their amphiploids and of the fertility of the latter, but did not report on their morphological constancy. Consequently, it is not possible to classify the amphiploids accurately at this writing.

Three of the new combinations, however, are of special interest from the biosystematic point of view. One, *Aegilops speltoides* Tausch, $n = 7$, × *A. sharonensis* Eig, $n = 7$, had homologous chromosomes with almost complete pairing in the undoubled F_1, and pollen 12 per cent fertile. The parents are evidently distinct ecospecies of one cenospecies. For these reasons their amphiploid, which was only 25 per cent fertile, should be classified with *Primula kewensis* and *Aquilegia Janczewskii* and be expected to segregate.

Only two combinations had essentially nonhomologous chromosomes. It is a fairly safe conclusion that these hybrids are inter-cenospecific. One of these was *Triticum monococcum* L., $n = 7$, × *Aegilops uniaristata* Vis., $n = 7$. The amphiploid was almost regularly 14-paired, with a fertility of 94 per cent, the highest in the entire group. Such an amphiploid should remain constant like *Phleum pratense* or *Triticale.*

The other was *Aegilops umbellata* Zhuk., $n = 7$, × *Haynaldia villosa* (L.) Schur., $n = 7$. This amphiploid broke down cytologically, for it had on an average less than 6 of the 14 possible pairs of chromosomes, with the rest remaining single. The irregular distribution of these broke up the parental genomes, and, as might be anticipated, the amphiploid was completely sterile. It would belong in a class with *Layia pentaglossa.*

In the remaining fifteen combinations reported by Sears, the parental chromosomes were partially homologous as is shown by the incomplete pairing, and the fertilities of the amphiploids varied between 13 and 86 per cent. It is to be expected that the most sterile of these amphiploid combinations would show interspecific segregation, and that the most fertile would remain essentially constant.

12. *Raphanobrassica*, $n = 18$; from *Raphanus sativus* L., $n = 9$, × *Brassica oleracea* L., $n = 9$ (Karpechenko, 1927, 1928). This amphiploid arose in the F_2 generation as a result of the fusion of diploid gametes of the F_1 hybrid.

Ninety F_1 plants resulted from this cross. These were diploid ($2n = 18$) throughout their structure. The chromosomes, however, failed to pair at meiosis, but sometimes went through a semi-heterotypic division resulting in unreduced sex cells, dyads, and other irregularities. The F_1 plants were allowed to pollinate openly on two different garden plots, both with seed cabbage adjacent, but one with and one without the possibility of backcrossing with radish. Although all 90 F_1 plants flowered abundantly the second year, only 19 were even slightly fertile. From these a total of 821 seeds were harvested.

The seed produced on the plot isolated from radish grew an F_2 of 361 plants, of which 229 were cytologically investigated. Of these, 213 were amphiploid ($2n = 36$), with normal meiosis and usually full fertility, although some were partially or completely sterile. The remaining 16 plants were classified as follows: 1 triploid, 11 hyperhexaploid, 1 hypopentaploid, and 3 hypohexaploid. All these were of reduced fertility. A morphologically uniform F_3 was grown from the amphiploids, and 32 plants investigated all had $2n = 36$ chromosomes.

The F_1 hybrids grown adjacent to radish yielded an F_2 population of 91 plants, which were almost all triploid and hypertriploid. The chromosomes of 73 of these were counted; 61 were triploid, $2n = 27$, and only 5 were amphiploid, $2n = 36$. Since only one triploid was obtained in that F_2 population from the garden isolated from radish, it appears certain in the present instance, from this and other evidence, that these triploids are the result of backcrosses between the F_1 hybrids and *Raphanus*, and also that there is very little backcrossing to *Brassica*.

A striking correlation was found in the *Raphanus-Brassica* hybrids between fruit structure and chromosome number. This made it possible to tell from gross appearance whether the hybrid was diploid, triploid, tetraploid, pentaploid, or hexaploid, and how the genomes of radish and cabbage were proportioned in it.

The hybrid between the radish and the cabbage was first produced by Sageret in 1826, and since then has been made a number of times. It was only in Karpechenko's masterful investigation, however, that the fertile amphiploid was produced. Since this is a stable new form, it has deservedly been given the name *Raphanobrassica*.

The radish and cabbage belong to distinct cenospecies of one comparium, since even in the backcross of the F_1 there are no effective means for an interchange of their genes. Thus they are somewhat more distantly related than *Triticum vulgare* and *Secale cereale*, mentioned above. The evolutionary significance of these thorough investigations and the conclusions to be drawn from them are pointed out under the following case.

13. *Brassica Napus* L., $n = 19$; from *B. campestris* L., $n = 10$, × *B. oleracea* L., $n = 9$ (Nagaharu U, 1935). This Linnaean species was synthesized both through somatic doubling and through unreduced gametes.

Several strains of each parent were crossed reciprocally. Out of more than 1100 flowers pollinated, only 33 seeds and 4 F_1 plants were obtained (these with *campestris* as the maternal parent), in addition to 15 individuals that apparently arose from induced self-pollination. The F_1 plants each had a different chromosome number: (1) $2n = 19$, a true F_1 with one genome from each parent; (2) $2n = 28$, a triploid with an extra *oleracea* genome; (3) $2n = 29$, a triploid with an extra *campestris* genome; and (4) $2n = 38$, an amphiploid with duplicated genomes of each parent. This amphiploid closely resembled *B. Napus*, and its chromosomes produced 19 pairs during meiosis, although occasionally 2 or 4 chromosomes failed to pair. It was exceedingly fertile, and since no evidence of diploid gametes could be found, it was supposed to have arisen by somatic doubling soon after fertilization.

The true F_1, with 19 somatic chromosomes, was also morphologically similar to *B. Napus* but highly sterile. Its meiosis was very irregular, with 0 to 8 loosely paired bivalents and 19 to 3 univalents. An F_2 generation from this plant consisted mainly of fertile amphiploids, having 38 chromosomes tightly paired at heterotypic metaphase. These plants, then, were considered to have arisen through the fusion of diploid gametes. By both methods, U considered that *B. Napus* had been artificially produced.

Brassica Napus, the kohlrabi, swede, or rape, occurs as a grainfield weed in Europe, western Asia, and North Africa, but no other wild types of it are known. One form is used as a vegetable and as livestock feed, and another is a source of vegetable oil. The cabbage progenitor, *B. oleracea*, is native along the coasts of southern and western Europe. The wild form of the other progenitor, the turnip, *B. campestris*, is a troublesome grainfield weed throughout Europe and western Asia and has been introduced to most of the temperate parts of the world.

It is not known when or where *Brassica Napus* evolved. The name *Napus* dates back to Roman authors, but it is not definitely known whether it refers to this species or to a close relative. The kohlrabi is known to have been under cultivation in Bohemia in 1610, but since the plant is morphologically intermediate between its parents, it has at times been considered a variety of one or the other, or a hybrid between them. Consequently, its history is hard to trace. Since the progenitor species have overlapping distributions, there have been many opportunities for crossing between them, with subsequent conscious or subconscious selection by man of the amphiploid for preservation. It probably has arisen repeatedly from different varieties of cabbage and turnip.

The radishes, cabbages, turnips, rapes, and mustards constitute one of the most important groups of agricultural plants. Almost all their species have extensive polygeneric synonymies, indicating the extent of the uncertainties in the minds of those who have attempted to classify them from morphology alone. Biologically, the species of *Raphanus, Brassica, Eruca,* and *Sinapis* belong to one comparium. Japanese, East Indian, Russian, British, and Danish investigators have shown that their species are interlinked by sterile or partially fertile hybrids. Their true natural relationships would be more fittingly emphasized if all were considered to be members of one genus, *Brassica.*

Natural species of the *Brassica* comparium have 8, 9, 10, 11, 12, 17, 18, or 19 pairs of chromosomes. Two additional artificial species have 27 and 29 pairs, respectively. All these numbers are dysploid, but they separate obviously into three distinct levels that correspond to the diploids, tetraploids, and hexaploids of polyploid species.

By analyzing the chromosome homologies in F_1 hybrids, Morinaga (1934) demonstrated that there are three basic genomes in *Brassica* from which those of the tetraploids could be obtained by addition. These belong to *Brassica nigra* Koch (= *Sinapis nigra* L.), $n = 8$; *B. oleracea* L. (including *B. alboglabra* Bailey), $n = 9$; and *B. campestris* L., $n = 10$. The last named includes *B. Rapa* L., *B. chinensis* L., *B. pekinensis* Rupr., and *B. japonica* Sieb., which prove to be but subspecies of it, for they all have 10 pairs of chromosomes, and the hybrids between them are completely fertile and have vigorous offspring. Morinaga's analysis indicated that on the tetraploid level *B. carinata* A. Braun, $n = 17$, was composed of a *nigra* and an *oleracea* genome; that *B. juncea* Coss., $n = 18$, had a *nigra* and a *campestris* set; and that *B. Napus* L., $n = 19$, had one set each from *oleracea* and *campestris.* U's subsequent synthesis of *B. Napus* from just these two parents was therefore a striking confirmation of the validity of Morinaga's work.

A general representation of the situation in the *Brassica* comparium is shown in figure 84. On the diploid level, *Brassica nigra, oleracea,* and *campestris* behave as distinct cenospecies, for no direct interchange of genes is possible between them, and their chromosomes are nonhomologous. The evolution of the species on the tetraploid level, however, brought both diploids and tetraploids into one cenospecies, because the diploids are partially interfertile with their amphiploid derivatives.

Raphanus sativus, $n = 9$, of a distinct cenospecies, is another member of the comparium, for it crossed with *Brassica oleracea* to form the amphiploid *Raphanobrassica,* as described above. Furthermore, Terasawa (1932) has obtained fertile and cytologically regular amphiploids, with 19 pairs of chromosomes, from the cross *Brassica chinensis* (a form of *B. campestris*), $n = 10$, × *Raphanus sativus,* $n = 9$. Orthodox taxonomists would include both of these in *Raphanobrassica,* although this artificial genus is composed only of artificial species.

Eruca sativa Coss. (= *Brassica verucastrum* L.), $n = 11$, and *Sinapis alba* L. (= *B. alba* Boiss.), $n = 12$, are other distinct cenospecies of the comparium, for U and his associates (1937) were able to hybridize both of them with *Brassica oleracea,* but the F_1 hybrids were completely sterile, and the chromosomes were essentially unpaired.

The evolution of this supergroup, then, has combined dysploidy and amphiploidy, with definite nodes or levels of ploidy. In addition to the diploid and tetraploid levels, a hexaploid level has been added to the structure, for Howard (1942) synthesized a hexaploid, $n = 27$, as an amphiploid of the *chinensis* form of *Brassica campestris,* $n = 10$, and *B. carinata,* $n = 17$. Similarly, the genomes of the already amphiploid *B. Napus,* $n = 19$, were added to that of *B. campestris,* $n = 10$, to produce *Brassica napocampestris,* $n = 29$ (Frandsen and Winge, 1932).

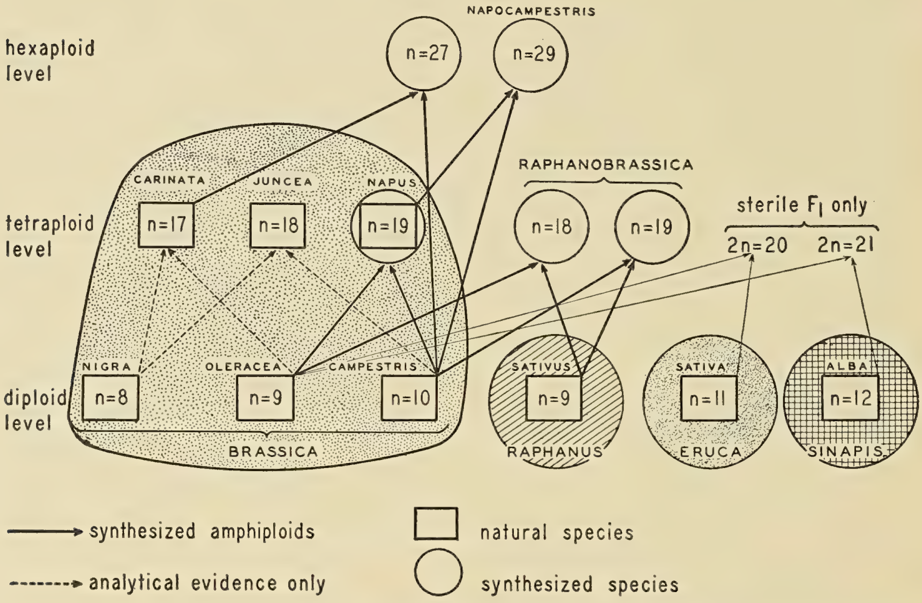

FIG. 84. Cytogenetic relationships in the *Brassica* comparium. Cenospecies indicated by shading. See text.

The natural species of the *Brassica* comparium compose four cenospecies, one of which has half a dozen ecospecies divided between diploid and tetraploid levels. If the artificial amphiploids that are known should arise spontaneously in the wild, the boundaries of the cenospecies might change to accommodate these new ecospecies. *Raphanus* and *Raphanobrassica,* however, would probably not be included in the same cenospecies with the Brassicas, because, as has been shown by Shchavinskaya (1937), the *Raphanus* genome is dropped out in all attempted backcrosses between *Raphanobrassica* and diploid *Brassica oleracea.*

Sufficient experimental work has been done in *Brassica* to give us the broader outlines of its rather complex evolutionary picture. Accordingly, it becomes an excellent example of the comparium, whose often intricate structure and development can be understood only after cytogenetic methods have revealed the simpler elements of which it is composed.

14. *Gossypium Thurberi* Tod., $n = 13$, × *G. arboreum* L., $n = 13$. An amphiploid with 26 pairs of chromosomes was produced somatically by Beasley (1940, 1942). This is one of a number of excellent investigations, conducted by various workers, that have greatly clarified the cytogenetic relationships between the species of cotton of the world.

Three species complexes within *Gossypium* are involved in Beasley's amphiploid: (1) a group of diploids having $n = 13$ chromosomes, occurring in both North and South America, including *G. Thurberi* (A. Gray) Tod., *G. Harknessii* Bdg., *G. Armourianum* Kearn., *G. aridum* (R. et S.) Skovs., and *G. Raymondii* Ulb.; (2) the cultivated diploid Asiatic species, also with $n = 13$ chromosomes, including *G. arboreum* L. and *G. herbaceum* L.; and (3) the cultivated American tetraploid species with $n = 26$ chromosomes, *G. hirsutum* L., *G. barbadense* L., and others.

Hybrids between the American *Thurberi* ($n = 13$) and the Asiatic *arboreum* ($n = 13$) are sterile, the chromosomes showing relatively few pairs of abnormal, attenuated appearance during first metaphase. When Beasley doubled the chromosomes of an F_1 hybrid by colchicine, he found the meiosis of the resulting tetraploid to be mostly regular, with 26 pairs. This amphiploid was highly female fertile, but most of its anthers were sterile. One flower, however, yielded an abundance of pollen, and so was used for selfing and for crossing reciprocally with the American tetraploid *hirsutum*, in each case developing seed. In the hybrids between the amphiploid and *hirsutum*, the chromosomes displayed mostly regular meiotic behavior with 24 or 25 pairs and only 2 or 4 univalents. In some pollen mother cells 26 pairs were found, but usually there were 1 to 3 or more multivalent associations.

These results are consistent with other facts concerning the cytogenetic relationships in *Gossypium*. The two Asiatic diploids, *arboreum* and *herbaceum*, are very closely related, for their hybrids have regularly paired chromosomes at meiosis and are partially fertile (Silow, 1941). The two cultivated American tetraploids, *hirsutum* and *barbadense*, likewise are interfertile and have homologous chromosomes (Webber, 1939). When these American tetraploids are crossed with either of the Asiatic diploid species, the resulting triploids have 13 pairs and 13 singles, showing that the American tetraploids have one genome in common with the Asiatic species (Skovsted, 1934; Webber, 1935, 1939). Furthermore, when either of the American tetraploids is crossed with one of the American diploids, *Thurberi, Harknessii, Armourianum, aridum,* or *Raymondii*, 13 pairs and 13 singles are found at meiosis, indicating that these, too, have one genome in common (Skovsted, 1937; Webber, 1939; Boza Barducci and Madoo, 1941). Finally, lack of pairing and presence of high sterility barriers between the Asiatic and American diploid species show that these two groups have different genomes (Skovsted, 1937; Webber, 1939).

By inference, this identifies one of the genomes of the American cultivated tetraploids as homologous with that of the Asiatic cultivated cottons, and the other as homologous with that of the wild American diploid cotton. Beasley, by combining the genomes of the Asiatic and American diploids and doubling them, produced the equivalent of the American tetraploid cotton. In other words, all these species may be considered as being composed of just two genomes.

The hypothesis advanced by Skovsted (1934) concerning the probable amphiploid origin of the American tetraploids from American and Asiatic diploid progenitors is thus upheld by conclusive evidence. Exactly what species or strains entered into the synthesis of the present-day cultivated American tetraploids is unknown, for the partial sterility of the hybrids between Beasley's artificial amphiploid and *G. hirsutum* shows that the genic balance in the synthesized product is not identical with that in the American tetraploids. The probability also exists that changes in the genic composition of *hirsutum* and *barbadense* have taken place since the original synthesis.

The diploid American and Asiatic species are so distantly related that when considered by themselves they belong to different cenospecies. The amphiploid American tetraploid cottons, however, have provided a bridge for an indirect exchange of genes between them (Harland and Atteck, 1941).

There are other genomes in the *Gossypium* comparium besides the two here identified. The chromosomes of the diploid Persian Gulf species, *G. Stocksii* Mast., are homologous with neither the Asiatic cultivated, nor the American diploid or tetraploid group. Beasley (1942) succeeded in obtaining a fertile amphiploid, with $n = 26$ chromosomes, from *G. Stocksii* ($n = 13$) and the Asiatic *G. arboreum* ($n = 13$). Other amphiploids may be expected between *Stocksii* and members of the American diploid group, and it is possible that higher levels of ploidy than are now known within the genus may eventually be synthesized by combining all three pairs of distinct genomes in one hexaploid species.

Before the era of the modern cytogenetic investigations in *Gossypium*, *G. Thurberi* and *G. aridum* were thought to belong to other genera, the former having been referred to as *Thurberia thespesioides* A. Gray, and the latter as *Erioxylum aridum* Rose et Standl. It is now clear that these two species not only are generically related to others in *Gossypium*, but belong to the same cenospecies as the American diploids, *Raymondii*, *Harknessii*, and *Armourianum*.

15. *Nicotiana Tabacum* L., $n = 24$; from *N. silvestris* Speg. et Comes, $n = 12$, × *N. tomentosiformis* Goodsp., $n = 12$ (Kostoff, 1938; Greenleaf, 1941, 1942; R. E. Clausen, 1941). Kostoff produced a fertile *Ta-*

bacum strain by a circuitous method of genome addition; Greenleaf and Clausen obtained partially sterile strains of the same by somatic doubling, using the callus method.

The F_1 hybrid of *N. silvestris* × *tomentosiformis* is very sterile, and its chromosomes are nonhomologous. Kostoff observed an average of 1.1 pairs in the meiosis of F_1. During a period of several years he backcrossed the F_1 with *silvestris.* Very few seeds resulted, but from them he obtained two triploids with 36 chromosomes. These were somewhat more fertile, but their meiosis was irregular. Many offspring were obtained when these triploids were backcrossed with *tomentosiformis.* Two of the progeny were straight amphiploids, containing two genomes from each parental species, and having 48 chromosomes. They were highly fertile and very much like *Tabacum.* Fertile hybrids between them and *Tabacum* segregated in a manner similar to varietal crosses of *Tabacum.*

Greenleaf and Clausen applied hetero-auxin to callus tissue of decapitated stems of the *silvestris-tomentosiformis* F_1 hybrid. This caused the chromosomes to double somatically. The resulting amphiploid was female sterile, owing to the arrested development of the embryo sacs at the 2- or 4-nucleate stage. The pollen was more than 90 per cent good, and was used for pollination of natural *Tabacum.* The resulting offspring were uniform, although only 17 per cent female fertile. When the Kostoff and the Greenleaf amphiploids were crossed, the hybrid had 24 pairs of chromosomes in meiosis, but only 13 per cent of the seeds were good.

Other species related to *tomentosiformis* were considered to be likely progenitors of *Tabacum.* These are *N. tomentosa* R. et P., *N. Setchellii* Goodsp., and *N. otophora* Griseb. All have 12 pairs of chromosomes and cross with *silvestris,* but when the resulting F_1 hybrids are somatically doubled, they show the same phenomenon of female sterility described above, except the hybrid with *otophora,* which rarely survives beyond the cotyledon stage.

The experimental results suggest that the genetic balance characteristic of *N. Tabacum* is difficult to obtain. Whereas the somatically doubled forms remain female sterile, the backcrossing of Kostoff's F_1 to first one and then the other of the parents resulted in compatible combinations. The natural variability in the parent species would permit slight alterations in the genome balance to occur in the amphiploid developed under such conditions. Strict gametic selection would be expected to eliminate unfit combinations and produce a successful form if that were possible.

These experiments indicate that the cultivated tobacco originated by the addition of the chromosomes of *silvestris* to those of a member of the *tomentosa* cenospecies, possibly *tomentosiformis.* Kostoff points out that *silvestris* and *tomentosiformis* are found in contiguous areas in northern Argentina and southern Bolivia, and therefore this is the most probable region of origin of *Tabacum.* This synthesis must have taken place long before the discovery of the American continents by Europeans, for in pre-Columbian times *N. Tabacum* was widely cultivated in, but apparently confined to, the West Indies and northern South America, particularly the Orinoco Valley, far from the area in which *N. silvestris* is native (R. E. Clausen, in litt.).

16. *Nicotiana digluta*, $n = 36$; from *N. glutinosa* L., $n = 12$, × *N. Tabacum* L., $n = 24$ (Clausen and Goodspeed, 1925; R. E. Clausen, 1928). This amphiploid appears to have arisen through somatic doubling in the F_1 zygote of a single plant.

From the cross *glutinosa* × *Tabacum* only two F_1 plants were obtained, one of which was partially fertile, the other sterile. The fertile F_1 plant was selfed and yielded a uniform F_2 population of 220 plants. A number of these were examined and found uniformly to have a chromosome number of $n = 36$. This population was reasonably fertile, and backcrosses succeeded with the parental species. The reciprocal cross yielded 15 F_1 plants, all completely sterile, but almost identical in appearance with the amphiploids. These F_1 plants could not be backcrossed with their parental species.

This amphiploid, named *digluta* to express its origin, was observed through the F_5 generation. During this time it remained constant, although it showed some irregularities in the chromosomal distribution.

Among the Nicotianas that have 12 as a basic chromosome number, no natural species is known with a number higher than $n = 24$. Therefore, the 36-chromosome *N. digluta* represents a new level of polyploidy in the genus. It is genetically isolated from its *glutinosa* parent, with which it gave only highly sterile hybrids, but its hybrids with *Tabacum* were highly fertile. In the F_2 of the latter backcross, there is an extensive elimination of unpaired *glutinosa* chromosomes, resulting in a tendency to revert to *Tabacum.* Accordingly, if *digluta* were to arise in nature, it might maintain itself in association with *glutinosa,* because of the hybrid sterility between them; but it probably would be slowly eliminated in association with *Tabacum.*

Aside from this regression, the syntheses of *Nicotiana Tabacum* and *N. digluta* illustrate perfectly the theory of the evolution of polyploidy as presented by Winge (1917). The chromosomes of *silvestris* were added to those of *tomentosiformis* to produce the cultivated tobacco, *Tabacum,* and this species, in turn, combined its genomes with that of *glutinosa* to produce the hexaploid *digluta.* Thereby the polyploid series $n = 12$, 24, and 36 was experimentally produced through the successive addition of the genomes of three diploid species belonging to distinct cenospecies. This process, however, merged them into one cenospecies, permitting a limited interchange between genomes through the amphiploids as intermediaries.

SEGREGATING NICOTIANA AMPHIPLOIDS. Additional amphiploids of *Nicotiana* have been reported by several authors. One of these is *Nicotiana rupa*, $n = 36$, which arose from *N. rustica* L., $n = 24$, × *N. paniculata* L., $n = 12$ (Lammerts, 1931, 1932; Kostoff, 1935). This hybrid combination was one of the many studied by Kölreuter (1763). Goodspeed, Clausen, and Chipman (1926) found that the F_1 has 12 pairs and 12 single chromosomes, indicating that 12 of the *rustica* chromosomes are probably homologous with the 12 from *paniculata*. The undoubled F_1 is slightly fertile on selfing and moderately fertile on backcrossing. Most of the F_2 offspring obtained by Lammerts—namely, 30 out of 37 plants that flowered—was amphiploid, $2n = 72$. The other 7 had chromosome numbers ranging between $2n = 38$ and 58, and the pairing in these shows that the parental genomes are not inherited intact, but that some interchange takes place.

Both Lammerts and Kostoff found that the amphiploids were uniform in certain characters but variable in others, such as habit, and detail of leaf and flower shape. Their fertility was moderately good, although some individuals were fairly sterile. The pairing was not regular, because there were more than two chromosomes of a kind. This made interchanges possible between the parental genomes, and Kostoff points out that as a result pseudo amphiploids arose. Lammerts carried *Nicotiana rupa* to the F_7 and found that the segregations continued for several generations, although gradually the five individual lines became uniform and distinct from one another. Even in the F_7, however, considerable chromosomal irregularity remained, for 11 per cent of the plants had aberrant chromosome numbers.

Nicotiana rustica and *N. paniculata* are remotely related ecospecies of one cenospecies, as is shown by the behavior of the undoubled F_1. The segregations of the derivative, *N. rupa*, and the homology of the *paniculata* chromosomes with one of the *rupestris* sets, would place this amphiploid, in our classification, with *Primula kewensis* (table 12).

A similar case was reported by Kostoff (1935) in the amphiploid *Nicotiana Vaviloviana*, $n = 21$, synthesized from *N. glauca* R. Grah., $n = 12$, × *N. Langsdorffii* Schrenk, $n = 9$. The undoubled F_1 has 9 pairs of chromosomes and 3 singles, and the amphiploid shows a greater segregation than *N. rupa*. Kostoff emphasized the point that the pseudo amphiploids had a low fertility, whereas the straight amphiploids were almost fully fertile.

An amphiploid of Solanum. An amphiploid with approximately 60 pairs of chromosomes was produced somatically by decapitation of the hybrid *Solanum luteum* Mill., $n = 24$, × *S. nigrum* L., $n = 36$ (Jörgensen, 1928). The parents are members of the cosmopolitan *S. nigrum* complex, the wild forms of which have the chromosome numbers $n = 12$, 24, and 36. *Solanum luteum* is a Mediterranean species, which some authors consider to be only a variety of *nigrum*. The pollen of the undoubled F_1 hybrid was only 8 per cent fertile, and the hybrid was sterile on selfing, but slightly fertile in backcrosses with either parent. Its chromosomes paired well, showing 24 pairs and 12 singles, but half the pairs could have been *nigrum* chromosomes pairing inter se, for haploid *nigrum* was observed to have approximately 12 pairs and 12 singles. Some tetravalent chromosomes were found in the amphiploid, and its pollen was only 35 per cent fertile.

At present, no further data on the behavior of this amphiploid are available, and so it cannot be classified with assurance. Its chromosome number, $n = 60$, is much higher than that of any wild species of *Solanum*, which does not exceed $n = 36$. Evidently its parents are distinct ecospecies of one cenospecies, with basically similar genomes. Unless there is preferential pairing between the chromosomes from one parent, some segregation with fairly deleterious effect should be expected in later generations. The low pollen fertility of the amphiploid is indicative of aberrations, and no more than moderate success could be expected from such an amphiploid.

17. *Madia nutrammii*, $n = 17$; from *M. nutans* (Greene) Keck, $n = 9$, $\times$ *M. Rammii* Greene, $n = 8$. This amphiploid, reported in detail in chapter II, arose twice from unreduced gametes in hybrids of slightly different origin. Some amphiploids appeared in the F_2 generation, others in the F_3. They have been studied through the F_5.

Two F_1 hybrids, but no reciprocals, were obtained by controlled pollination. These F_1's had 17 somatic chromosomes, which were nonhomologous, only from 0 to 3 very long, drawn-out pairs being observed. The chromosomes did not always disjoin, but remained together until second metaphase, when all 17 divided equationally. Dyads resulted from this mode of division. The F_1 plants displayed some characters from each parent and were intermediate with respect to others. Both were highly sterile. Some 2000 seeds were harvested from them after open pollination, most of which were empty. Only 7 F_2 plants were obtained, which were classified as follows: 1 triple hybrid with the full genomes of three distinct cenospecies; 4 amphiploids or near amphiploids; and 2 triploids. The amphiploids could have arisen only by self-pollination induced by the presence of foreign pollen, and at least one of the triploids originated in the same way.

An isolated group of 5 plants, consisting of 3 tetraploid and 2 triploid individuals, with an aggregate fertility of 5.5 per cent, produced the seed for an F_3 by open pollination. The 220 F_3 plants were uniform, and morphologically very similar to the F_1 and F_2. Of a sample of 12 plants, 11 were fully amphiploid, with $2n = 34$, and 1 had $2n = 33$ chromosomes. Slight cytological irregularities were observed in some of the amphiploids up to the F_4 generation, such as the occasional failure of partners of one or two pairs to conjugate. This did not impair the fertility of the new species materially, however, and in the F_4 full fertility was attained.

The parental species are rare monotypic cenospecies of one comparium. Their natural distributions are now well separated, so there is no opportunity for *Madia nutrammii* to arise in the wild. Such an occurrence might have served to rejuvenate the parental monotypic cenospecies, saving them from a probable early extinction.

18. *Madia citrigracilis* Keck, $n = 24$; from *M. gracilis* (Sm.) Keck, $n = 16$, × *M. citriodora* Greene, $n = 8$. This amphiploid, reported in chapter III, arose three times from considerably different stocks. In each instance it appeared in the F_2 generation through the union of unreduced F_1 gametes. In this case the amphiploid was deliberately made in order to test the supposed hybrid origin of a recently discovered hexaploid species.

In the artificial cross only one F_1 hybrid was obtained, but three more were found in two spontaneous crossings. This hybrid combined characters of both parents, but on account of dominance, its floral characters were much closer to those of *gracilis*. Its chromosome number was $2n = 24$. Only 1 to 3 very long pairs were observed in meiosis, and many pollen mother cells contained no pairs. In such cells all the chromosomes stayed together until second metaphase, when an equational division resulted that had much the appearance of a first metaphase. Dyads were common among the tetrads. The fertility of the F_1 of the controlled cross was approximately 5 per cent, which was remarkably high for a hybrid between species so remote.

The single selfed F_1 hybrid produced 94 apparently good akenes, from which an F_2 of 47 plants was raised. Of these, 39 were amphiploid ($2n = 48$), and the remaining 8 were near amphiploid ($2n = 43$–47). The fertility of the F_2 plants varied between 4.5 and 69 per cent. They were uniform and similar to the F_1 and natural *citrigracilis*. The amphiploids were regularly 24-paired, or occasionally 23-paired with 2 singles. In the native *citrigracilis* 2 single chromosomes could also be found, although more rarely. The new amphiploid was constant in the F_3.

Madia gracilis and *M. citriodora* would belong to distinct cenospecies if the natural amphiploid *citrigracilis* and other high polyploid forms of *Madia* did not exist. The parental species grow adjacent to each other at perhaps several places in the wild, but their natural amphiploid is known only from a limited region.

AN AMPHIPLOID IN LACTUCA. Another amphiploid from the Compositae deserves mention because the understanding of it is much enhanced by an excellent cytogenetic analysis of the genus. Its final placement in table 12, however, would require data from generations later than those now on record. This amphiploid is *Lactuca indicata*, $n = 26$, which arose from unreduced F_1 gametes of *L. spicata* (Lam.) Hitchc., $n = 17$, × *L. indica* L., $n = 9$ (R. C. Thompson, 1942). The parents are native to the eastern United States and eastern Asia, respectively.

Fifteen F_1 plants were obtained from the initial cross, 5 of which were grown in a field at Beltsville, Maryland. Three of these were partly fertile, especially one plant which yielded several hundred seeds. From this plant 127 nonsegregating F_2 progeny were obtained, presumably all amphiploids. The F_2 plants resembled the F_1 in being intermediate between the parent species. The leaves were thicker in the amphiploids than in the F_1 plants, however, an effect apparently due to doubling of the chromosomes.

Root-tip preparations of the F_1 hybrids showed $2n = 26$ chromosomes, and meiosis in pollen mother cells was irregular. Often 8 bivalents, 1 trivalent, and 7 univalents were found, and lagging chromosomes, irregular tetrads, and sterile pollen were also characteristic features. In most of the cells, however, no pairing occurred during the first meiotic division. This was followed by an equational division, producing dyads. Sometimes no division took place, so that single giant microspores were formed which presumably also contained 26 chromosomes.

The F_2 plants mostly had 26 pairs of chromosomes in pollen mother cells. Quite frequently, however, lagging chromosomes were found, and occasionally, also, dyads, indicating some cytological instability. The evidence, therefore, strongly indicates that the amphiploids arose from the fusion of diploid gametes. Tests on the setting of seed in the F_2, which is the last generation as yet reported, showed that the amphiploids are from 10 to 50 per cent fertile. The name for this amphiploid, *L. indicata*, is taken from those of the parents.

From a biosystematic point of view, the cytogenetic analysis of the lettuce genus, *Lactuca*, has not progressed far enough to permit a satisfactory interpretation of the natural relationships among all the species, although few genera have been more thoroughly crossed. However, the results from the interspecific hybridizations that involved twenty-one species or forms reported by Thompson, Whitaker, and Kosar (1941), Whitaker and Thompson (1941), and Thompson (1943) make clear some of the relationships within it.

There are at least two distinct comparia in *Lactuca* on the diploid level, and, in addition, some isolated species not yet connected with the others through hybrids. The smaller comparium of the two contains the cultivated lettuce, *L. sativa* L., and a few other species, all with 9 pairs of chromosomes. Very extensive crossing attempts have failed to produce hybrids between its members and any other *Lactuca*.

The parents of *Lactuca indicata* belong to the other comparium, which is very large and, on the diploid level, contains two major groups, one with 9 pairs of chromosomes, to which *L. indica* belongs, and the other with 8 pairs. So far as is known, all the diploid species are native to Asia, Europe, or Africa. The species in the eastern United States, to which *L. spicata* belongs, are all on the tetraploid level, with 17 pairs of chromosomes, but they cross with both the 8-chromosome species and the 9-chromosome members of the *indica* group, producing sterile hybrids. Before these facts were known, Babcock, Stebbins, and Jenkins (1937) suggested that the American species arose through amphiploidy from 8- and 9-chromosome progenitors. Further indirect evidence supporting this hypothesis is the observation that there are up to 9 pairs of chromosomes in hybrids between the American species and certain 9-chromosome members of the *indica* group, indicating that one of the sets of chromosomes in the former is homologous with that of the latter.

The synthesis of the 26-chromosome *L. indicata* from *spicata* × *indica* initiates a new and higher level of ploidy in *Lactuca*. It did not, however, add a new genome to the structure of the American species, and the species is therefore not truly tri-genomic, but corresponds to *Brassica napocampestris.*

THE PLUM AS A POSSIBLE AMPHIPLOID. *Prunus domestica* L., which includes both the cultivated plums and prunes, has 24 pairs of chromosomes, a higher number than is known in any of the wild species in its group. Its closest relatives are *P. cerasifera* Ehrh., the cherry plum, with 8, and *P. spinosa* L., the blackthorn, with 16 pairs of chromosomes. Crane and Lawrence (1931) suggested that the cultivated plum may have arisen as an amphiploid from hybrids between these or related species. Some confirmation of this theory has come from investigations by Rybin (1936) on experimental and spontaneous hybrids in the Caucasus between *P. cerasifera* ssp. *divaricata* (Ledeb.) C. K. Schneid. and *P. spinosa.* The natural hybrids are triploid and resemble forms of the cultivated plum, but they are very highly sterile, although six or seven fruits were obtained from two trees that flowered abundantly. In the artificial crossing of these two species, Rybin obtained 16 F_1 seedlings from about 9000 flowers. One of these was the desired amphiploid, with $2n = 48$ chromosomes, and the others were triploids. It is not known whether the amphiploid originated from somatic doubling after fertilization or from the chance union of a diploid ovule of *cerasifera* with a diploid pollen of *spinosa*, but the former appears the more likely.

The chromosomal behavior of the triploid F_1 was not reported, because the plants had not reached the flowering stage. Darlington (1930) found that in the hybrid between diploid *cerasifera* and hexaploid *domestica*, there were 16 pairs of chromosomes, indicating autosyndesis between two of the genomes from *domestica* and homology between the third and the one from *cerasifera.* Despite the good pairing, this hybrid is highly sterile except when pollinated by its *domestica* parent. Other hybrids between diploid *Prunus* species have up to 8 pairs, indicating a high degree of homology between their chromosomes. Therefore, if *domestica* is an amphiploid from *cerasifera* and *spinosa*, this homology should result in considerable segregation. There are distinct differences in the color of the pulp and skin and in the flavor of the fruits of the putative parents. The cultivated plums have an infinitely wider range of color, size, and flavor than either of these. Many of the segregants are successful under cultivation, although *P. domestica* is not known to have established itself anywhere in the wild in spite of the occurrence of the natural triploid hybrids.

AMPHIPLOIDS IN THE GENUS RUBUS. The blackberries and the raspberries belong to one of the most complex plant genera investigated. It is the blackberry section of the genus, however, that is most difficult taxonomically. Hundreds of species have been named among the blackberries, many of them asexually propagating apomictic clones.

All species of *Rubus* follow a 7 series in their chromosome numbers, and several experimental amphiploids are known, but before these are discussed it is necessary to summarize the biological situation in the better-known groups of the genus.

The North American and European species appear to fall within some four, or possibly even three, sections. Some authors call these subgenera, but the numerous hybrids between them indicate that biologically all belong in one comparium. Most of the complexity is found within the section *Eubatus*, the blackberries and dewberries. The three other sections, *Cylactis* in its widest sense, *Idaeobatus*, the raspberries, and *Anoplobatus*, the flowering raspberries, are much less polymorphic.

The section *Cylactis* contains the herbaceous species, which generally have palmately lobed, palmate, or ternate leaves. Its members are north-circumpolar or alpine in distribution. Their cytological situation is not well known, but *Rubus Chamaemorus* L., the bakeapple or cloudberry, a characteristic circumpolar bog plant, is octoploid ($n = 28$), and *R. saxatilis* L., the European stoneberry, is tetraploid, and forms natural hybrids with the diploid *R. arcticus* L., of its own section, and also with *R. idaeus* L., $n = 7$, of the section *Idaeobatus*, and *R. caesius* L., $n = 14$, of *Eubatus* (Vaarama, 1939). The section *Cylactis* appears to attain its greatest diversity in North America.

The sections *Anoplobatus* and *Idaeobatus* seem to be closely related. Both include more or less erect bushes with berries that fall from the receptacle, or core, but the members of *Anoplobatus* have palmately lobed leaves and unarmed, woody stems, whereas those of *Idaeobatus* have pinnate leaves and herbaceous stems with weak prickles. The thornless flowering raspberries of the section *Anoplobatus* are North American and East Asiatic, and include the species pair *R. odoratus* L. and *R. parviflorus* Nutt., both of which are diploid. One supposed triploid is known in this section, *R. deliciosus* Torr., the Rocky Mountain flowering raspberry.

All the true raspberries, section *Idaeobatus*, are diploid, with the exception of some cultivated tetraploid varieties. North America again is the center of greatest diversity for this section. Only the European cultivated raspberry, *R. idaeus* L., is native in Europe, whereas outstanding raspberries in North America include *R. strigosus* Michx., the American red raspberry; *R. occidentalis* L., the eastern blackcap, with its close relative *R. leucodermis* Dougl. in the far west; *R. neglectus* Peck, the purple-cane raspberry; and the very distinct salmonberry, *R. spectabilis* Pursh, of the northwest coast. Within each of these two sections the barriers to interbreeding appear to be slight, and great morphological diversity has developed without changes in chromosome number.

The section *Eubatus* consists of forms with drupelets that adhere to the fleshy receptacle, or core, and with leaves that are usually compound. The berry, including the receptacle, detaches from the calyx. Those species with strong, erect or arching, woody stems are the true blackberries or brambles, and those with weaker, more or less trailing and rooting stems are the dewberries. In contrast with the raspberries, the *Eubati* form one of the most intricate polyploid complexes known, although the chromosome numbers for the most part are not very high.

In Europe only 2 diploid blackberries have been found, namely, *R. ulmifolius* Schott (including *R. rusticanus* E. Merc.) and *R. tomentosus* Borkh., but in addition 3 triploids, 80 tetraploids, 18 pentaploids, 18 hexaploids, and 1 heptaploid are known. Most of these European polyploids are facultatively apomictic (Lidforss, 1914; Gustafsson, 1939), a fact which accounts for the extreme multiplication of forms.

Because of the confused synonymy, it is much more difficult to gain an impression of the cytological situation in the blackberries of eastern North America. When information from Longley (1924), Yarnell (1936), and Darrow (1937) is pooled, it appears that there are at least 6 recognized diploid species ($n = 7$), namely, *R. allegheniensis* Porter, *R. argutus* Link, *R. canadensis* L., *R. setosus* Bigel., *R. cuneifolius* Pursh, and *R. trivialis* L. Taking the synonymy, also, into consideration, Longley listed in addition 12 triploids, 2 pentaploids, and 2 hexaploids. Most of the polyploids were more or less pollen sterile and probably hybrids. The absence of tetraploids is rather startling. Darrow records 2 wild tetraploids from Maryland as probable hybrids, and the 17 cultivated ones he lists are also generally considered to have arisen by hybridization.

Darrow (1937) has taken up Brainerd's suggestion (1906) that the wild blackberries of the eastern United States, like the violets, belonged to few species when the country was first settled. Then, with the removal of the forests and the clearing of the land, they spread; previously isolated species came together, and opportunity was given for extensive hybridization, with the resultant remarkable multiplication of forms.

Brainerd and Peitersen (1920) and Peitersen (1921) showed that many of the so-called species of New England were recent hybrids, because (1) they were intermediate between other species, (2) they usually grew in association with them, (3) their pollen was highly sterile and fruit setting was often poor, (4) they segregated strongly when inbred, the segregants in some cases resembling the putative parents, and (5) they could be synthesized by crossing the suspected parents. Consequently, at least a number of American *Rubi* are normally sexual. But the widely distributed swamp dewberry, *R. hispidus* L., can scarcely be sexual, for Peitersen found that it has only 10 per cent fertile pollen, and Longley reports it is pentaploid.

The fact that no nonhybrid wild tetraploid blackberries have been discovered in the eastern United States suggests that at the time of settlement the blackberry flora of this area possibly consisted mainly of diploid species. The presence of low to moderate fertility in the present-day wild hybrids may mean that their parents were ecospecies of one cenospecies. The occasional production of diploid gametes in connection with hybridization would result in triploids and tetraploids. Crane and Darlington (1927) concluded from breeding experiments that such gametes occurred in a European diploid *Rubus*. Similarly, doubling of the chromosomes in the triploids might account for the rare hexaploids. Such a pattern of origin should explain the multiplicity of forms described in these eastern blackberries, where only a few forms were found to be fully pollen fertile.

There is no geographic connection between the eastern North American blackberries and those of the Pacific states, for the Rocky Mountains are devoid of members of the section *Eubatus*. This makes the derivation of the western blackberries from the eastern (Brown, 1943) very hypothetical.

In contrast with the blackberries of the eastern United States and Europe, those of the Pacific states are highly polyploid. These are the dewberries of the *R. ursinus* Cham. et Schlecht. complex, with very long, trailing stems. Brown (1943) found chromosome numbers of $2n = 42$, 56, 63, 70, 77, and 84 in the wild forms, with 56 and 84 by far the most common. The absence of diploids and lower polyploids in this complex is striking.

A series of confusions has obscured the taxonomy of the Pacific blackberries. This caused Brown to overlook the true status of the long-lost *R. vitifolius* Cham. et Schlecht., which he redescribed as a new species, *R. lemurum*, and placed in the east Asiatic section *Chamaebatus*, probably on account of its similarity to *R. nivalis* Dougl., which Bailey (1941) had treated as a member of that group. These species have shallowly lobed simple leaves, in contrast with *R. ursinus* and other members of the complex, with their compound leaves. Both *vitifolius* and *ursinus* were described from material collected at the same time at San Francisco (Bailey, 1925, pp. 218–220).

The thorough cytotaxonomic investigations of Brown (1943), which unfortunately did not include *nivalis*, a rare montane species of western Oregon, Washington, and British Columbia, make it clear that three basic types can be recognized within the *ursinus* complex in addition to some secondary hybrid derivatives. From these studies, but not strictly following Brown's classification, the following biological units stand out:

(1) On the coastal bluffs of central California from at least Fort Bragg in the north to Point Sur in the south is the rare *R. vitifolius* C. et S. (*R. lemurum* S. Brown), a duodecaploid with 84 somatic chromosomes.

(2) In the Coast Ranges and the Sierran foothills from southern Oregon to southern California is the common California dewberry, *R. ursinus* C. et S., an octoploid, with $2n = 56$; its leaves are typically 3-foliolate.

(3) From northern California to British Columbia grows another duodecaploid species, *R. macropetalus* Dougl., $2n = 84$, typically with 5-pinnate leaves and glandular inflorescence.

The following forms appear to be of secondary importance. A rare, probably relict, hexaploid form, $2n = 42$, was discovered in northern California. Like 84-chromosome *macropetalus*, of which this is a possible ancestor, it has 5-foliolate leaves, but the inflorescence is not glandular. Forms with $2n = 70$ chromosomes are found in two regions: one, where the 84-chromosome *vitifolius* meets the 56-chromosome *ursinus* in central coastal California; the other, where *ursinus* intermingles with the 84-chromosome *macropetalus* in northern California in company with the 42-chromosome relict form. This intermediate chromosome number and much morphological recombination point to hybrid swarms in these two areas, which should be a deterrent to placing *vitifolius* and *ursinus* in different sections or subgenera. Of very minor importance in the flora are forms with $2n = 63$ and 77 chromosomes.

The Pacific blackberries evidently form a characteristic polyploid cenospecies with at least three cytologically, ecologically, and morphologically distinguishable ecospecies, although many hybrid intermediates occur where these meet. The high polyploidy should permit these intermediates to be fairly successful. The 56-chromosome *ursinus* is a warm-temperate ecospecies of the Upper Sonoran and Humid Transition zones in California; the 84-chromosome *macropetalus* is a cooler-temperate ecospecies of the Transition zone from Oregon to British Columbia; and, finally, the 84-chromosome *vitifolius* is a maritime ecospecies of central California.

Possibly *Rubus nivalis* also belongs to this cenospecies. It occupies an ecologically distinct montane zone from Oregon to British Columbia and northern Idaho, and differs from *vitifolius* mainly in its much wider stipules, smaller petals, and much less robust growth. Neither of these species flowers very freely, and they appear to be relicts. In leaf form they somewhat resemble *R. Chamaemorus* L., *R. stellatus* Sm., and *R. lasiococcus* A. Gray, of the section *Cylactis*, but in other characters they belong to *Eubatus*.

The origin of this unique polyploid complex cannot be fully visualized at present, because no trace has been found of forms on the lower polyploid levels that necessarily must have preceded its members. Some early progenitors may have combined genomes of species belonging to different sections. The flowers of this complex are dioecious like those of *Chamaemorus*, but these high polyploids are evidently still largely sexual, in contrast with the European apomicts of much lower chromosome numbers. The great economic importance of this Pacific coast complex is due to the fact that it is highly crossable with members of other sections and has contributed to some of the most important cultivated blackberry varieties. This crossability is possibly facilitated by some of the forms' having essentially an autoploid structure on top of an amphiploid one, as is suggested by the multivalent associations of chromosomes in its species (Thomas, 1940; Brown, 1943).

THE NESSBERRY. This is one of the earliest amphiploids. It arose in 1918 from the cross *Rubus trivialis* Michx., $n = 7$, × *R. strigosus* Michx., $n = 7$, the southern dewberry and the American red raspberry, respectively, in a series of very methodical and well documented investigations reported upon by Ness (1921, 1925). Longley and Darrow (1924) found that the nessberry had 14 pairs of chromosomes, but its true nature was not detected until later (Yarnell, 1936). This amphiploid appears to have been generally overlooked in the literature. Ness pointed out that his results did not agree with simple Mendelian inheritance, but he presented the facts so lucidly that amphiploidy is at once suggested.

The 21 F_1 plants of *trivialis* × *strigosus* resembled mostly the raspberry parent. They were highly sterile and produced no viable seed when caged, but from open pollination enough seed was obtained to yield an F_2 of 280 plants, of which 125 survived to bear fruit the following year. Various types appeared in this F_2, some of which were undoubtedly backcrosses to the dewberry parent, but among a group with raspberry characters, 5 very vigorous and highly fertile plants stood out. From 3 of these a nonsegregating and fertile F_3 of 859 individuals was grown. The nessberry was propagated vegetatively from 8 selected F_3 plants. By 1925, 5653 plants had been distributed by the Texas Agricultural Experiment Station.

Ness clearly called attention to the fact that the variations observed in the new form and its crossing behavior were like those of a good species. The nessberry resembles a raspberry of excessively strong growth; its fruit is deep blood red, mildly acid, and strongly reminiscent of raspberry in flavor. Its greatest disadvantage lies in the difficulty in picking it, for the drupelets adhere to the core as in the dewberry, and the core, in turn, adheres to the calyx as in the raspberry. The calyx is therefore picked with the fruit as in the strawberry. Compensating assets, however, are its drought resistance, inherited from the southern dewberry, in combination with the raspberry flavor, for, as is pointed out by Ness, the raspberries are short-lived in Texas and likely to die before fruiting. Thus by adding the genomes of the raspberry to those of the native dewberry, it was possible to transfer the raspberry flavor to a new species, the nessberry, adapted to the South.

Evidently Ness produced a second and related amphiploid, although no cytological investigation was made of it. The cross, *R. trivialis* Michx., $n = 7$, × *R. neglectus* Peck, $n = 7$, the purple-cane raspberry, produced 21 very sterile F_1 hybrids. A small F_2 of 19 strong, very uniform plants was obtained from these, similar to the F_1 except that their flowers were larger and fertile. The growth of this new, constant form, however, was too rank for the amount of fruit obtained, and so it was discarded.

The two species that were combined with *trivialis* to produce amphiploids, namely, *strigosus* and *neglectus*, are considered to be closely related, and their genomes must be very similar. This point is emphasized by the fact that Ness produced another fertile hybrid by pollinating the *trivialis* × *neglectus* F_1 with the nessberry. Seven plants of this secondary hybrid were obtained, all vigorous, fully fertile, and nonsegregating, for from them a fertile F_2 of 279 uniform plants was raised. To be fertile, the 7 plants of (*trivialis*, $n = 7$, × *neglectus*,

$n = 7$) × nessberry, $n = 14$, must have arisen from diploid ovules. They should contain 7 pairs of dewberry and 7 pairs of raspberry chromosomes, one set of the latter from *neglectus* and the other from *strigosus.* The slight differences between the genes of the two raspberries would probably not be noticed in the presence of two *trivialis* genomes, hence the apparent constancy.

A notable quality of the nessberry that was emphasized by Ness is that it lent itself with ease to further hybridization with other forms. He backcrossed it with its dewberry parent, and crossed it with the European raspberry, *R. idaeus* L., and with blackberries. Although most of the crossings appear to have been made with diploid species, and the F_1 hybrids usually were highly sterile, fertile tetraploids appeared in later generations of these crossings (Yarnell, 1936), possibly as a result of conscious selection of forms resembling the nessberry but with better picking qualities.

The *trivialis* parent of the nessberry belongs to the section *Eubatus,* and the *strigosus* parent to *Idaeobatus.* Nobody has recorded observations on the pairing of chromosomes in hybrids between diploid members of these sections, and so the homology of their chromosomes is not known. Longley and Darrow (1924) found regular pairing in the nessberry, but Yarnell's figure of meiosis suggests some quadrivalent association among its chromosomes, although the figure is lacking in detail. This author also noticed quadrivalents in most of the tetraploid descendants of its crossings with diploid species, but the figures are not convincing. For this reason the nessberry cannot with full confidence be placed in table 12, although it probably belongs in the lower right-hand box.

THE AMPHIPLOID ORIGIN OF RUBUS MAXIMUS. Rozanova (1934, 1938) has produced *R. maximus* Marsson, $n = 21$, from *R. idaeus* L., $n = 7$, × *R. caesius* L., $n = 14$; it therefore belongs among the rare cases of resynthesis of a wild species. The chromosome number of the wild *maximus* is as yet unknown, but Rozanova produced an amphiploid which duplicated it in morphology and fertility. This amphiploid is the European parallel to the nessberry, in so far as it combines the genomes of the European raspberry with those of the common European dewberry.

The European raspberry, *R. idaeus,* and the dewberry, *R. caesius,* hybridize in localities where they meet, and the hybrid backcrosses to the parents. In European floras these hybrid products are recognized under various names, but they are difficult to classify because *caesius* crosses also with the true blackberries. The primary hybrids, backcrossings, and suspected amphiploids of the *idaeus-caesius* combinations constitute the series *Subidaei* within the section *Eubatus.* Whereas *idaeus* is a sexual species, *caesius* is facultatively apomictic, according to Lidforss (1914). Most of the units in the series *Subidaei* are more or less sterile because they are remote interspecific hybrids, and, since apomixis is a recessive character, they generally require sexual propagation. *Rubus maximus*, however, is one unit of the *Subidaei* which is fertile. It has been recognized from northeastern Germany, southern Sweden, and Denmark. It was first found in dune copses along the shores of western Pomerania, and this is the form that Rozanova has duplicated.

Rozanova pollinated *R. idaeus* with a race of *R. caesius* from Turkestan and obtained an almost sterile triploid F_1 ($2n = 21$), which produced only 2–6 per cent good pollen. The reciprocal combination failed. Restitution nuclei were observed during meiosis of the triploid hybrid, and the fertile gametes probably originated from such as these. Three F_2 plants were obtained, and these duplicated *R. maximus* morphologically. They were hexaploid ($2n = 42$), fertile, and probably constant. Spontaneous backcrossings of the F_1 with *idaeus* yielded moderately fertile plants with $2n = 28$ chromosomes, and, similarly, backcrossings with *caesius* produced comparatively fertile plants with $2n = 35$. The chromosome numbers of the offspring indicate that only unreduced gametes of the triploid F_1 functioned. If the *idaeus* genomes are indicated by II, and those of *caesius* by $C_1C_1C_2C_2$, their hybrid offspring should have the following constitutions:

F_1	IC_1C_2	$2n = 21$
Backcross to *idaeus*	IIC_1C_2	$2n = 28$
Backcross to *caesius*	$IC_1C_1C_2C_2$	$2n = 35$
Amphiploid, *maximus*	$IIC_1C_1C_2C_2$	$2n = 42$

Gustafsson (1939) actually found these chromosome numbers in wild plants of *idaeus* × *caesius* from Sweden. Likewise, he found $2n = 35$ in five forms of *R. pruinosus* Arrh. and in one of *R. Warmingii* G. Jens., and $2n = 28$ in four forms of *R. centiformis* Frider., all generally supposed to belong to the *Subidaei.* One plant of *centiformis*, also, and one of *idaeus* × *caesius* were found to be hexaploid, $2n = 42$, but it was not stated whether these hexaploids represented amphiploid combinations, nor whether they were fertile, as is *R. maximus.*

The high sterility of the F_1, the presence of restitution nuclei, and the fact that only those gametes were viable in the triploid F_1 that contained a full set of chromosomes suggest that the chromosomes of the European raspberry are nonhomologous with those of the European dewberry, although direct proof is lacking. Vaarama (1939) observed 4–7 (an average of 5) loose pairs in a spontaneous hybrid of this combination from the Åland Islands in the Baltic. This shows that the pairing is considerably reduced. Also, it is still unknown whether the pairing is between the *caesius* chromosomes themselves or between *caesius* and *idaeus* chromosomes. Certainly these species belong to distinct cenospecies of one comparium. For these reasons, *Rubus maximus* most likely falls within the main body of the amphiploids in the lower right-hand corner of table 12.

THE VEITCHBERRY. The veitchberry, $2n = 28$ (Crane and Darlington, 1927), is supposed to be an amphiploid between a raspberry and a true blackberry of the *Eubati veri.* One parent is thought to have been a form of the American red raspberry, *R. strigosus* L., $n = 7$, and the other the *rusticanus* form of the diploid southern European blackberry, *R. ulmifolius* Schott, $n = 7$. The color of the veitchberry is dark purple, intermediate between those of its probable parents. Also, it has twice as many chromosomes, but their pairing is not known because the anthers degenerate before meiosis. On account of its pollen sterility it was suggested that it propagates asexually by pseudogamy. If this is true, the veitchberry is an interesting case of an apomictic plant originating as a hybrid of two sexual species.

THE LOGANBERRY AND ITS RELATIVES. The origin and history of the loganberry (*R. loganobaccus* Bailey) is better known than that of most other cultivated plants. Nevertheless, the mechanism by which it arose and its true nature have been under much dispute. Recent investigations by Crane and Darlington (1927), Crane (1940*b*), Thomas (1940), and Brown (1943) have greatly clarified its origin.

The loganberry's morphological and genetical characteristics suggest that it is an amphiploid, but its chromosome number is intermediate between those of its putative parents. It is hexaploid, with 21 pairs of chromosomes; its parents, the California dewberry and the European raspberry, have 28 and 7 pairs, respectively. The investigations referred to above suggest that it arose from a dewberry genome with 28 chromosomes capable of forming 14 pairs, fertilized by a diploid raspberry genome with 14 chromosomes capable of forming 7 pairs. The undoubled F_1 hybrid would therefore amount to an amphiploid with 14 pairs of dewberry and 7 pairs of raspberry chromosomes, the only variation from normal amphiploidy being that the parental chromosomes were predoubled before crossing. This explains very simply why this undoubled

F_1, although an intersectional hybrid, remained fertile and relatively constant.

The loganberry originated in 1881 in Judge J. H. Logan's garden in Santa Cruz, California (Bailey, 1923). Logan collected fruits from plants of a cultivated race of the native dewberry, *R. ursinus* C. et S., and sowed them. These plants grew beside an old raspberry colony which had been there since about 1860. This raspberry was related to the Red Antwerp variety of *R. idaeus* L. Approximately 100 seedlings were set out, and when they fruited it was discovered that one was quite distinct from the others. This was thought to be a hybrid between the dewberry and the raspberry.

The California dewberry is a low trailing plant with prickly, very long canes, 3-foliolate leaves, and black fruit. Like many plants native to the California Coast Ranges, it is evergreen. The European raspberry is an upright deciduous shrub with 5-pinnate leaves and red fruit. The color of the berry in the suspected hybrid turns from a bright glowing red, like that of the raspberry, to dull purplish red at maturity; the drupelets are more compact than in the dewberry parent, but they adhere to the fleshy receptacle, which, unlike that of the nessberry, separates from the calyx. This added dewberry influence in the loganberry is possibly due to the fact that it has twice as many dewberry chromosomes as the nessberry without any increase in the number of raspberry chromosomes. The long, arching canes of the loganberry are stronger than those of the dewberry, but the spines are smaller. Its leaves, however, are 5-pinnate, and its flowers hermaphroditic like those of the raspberry—not dioecious as in all the Pacific berries. It is therefore evident that this hybrid combines the characters of two sections of *Rubus*, but it is fertile, and nonsegregating in regard to sectional characters.

One would expect that the common 56-chromosome *R. ursinus* would be the maternal parent of the loganberry. Crane and Darlington (1927) found that the loganberry had 42 somatic chromosomes, and Thomas (1940) reported that these normally form 21 pairs. According to Thomas, *R. ursinus* is less regular cytologically, for in addition to those chromosomes which pair regularly, some occur in groups of 4, as well as in groups of 3 and singly. Brown (1943) verified this, and, in addition, found a small percentage of groupings with 5 or 6 chromosomes. This suggests that *ursinus* contains several sets of more or less homologous chromosomes; a better pairing should, therefore, be expected in the loganberry, which contains only half of the *ursinus* chromosomes.

So far as is known, the tetraploid raspberries have originated since 1860, when Judge Logan's raspberry was planted. Probably, therefore, his raspberry produced occasional diploid pollen, from which the loganberry arose. Had his raspberry been tetraploid, more than the single hybrid should be expected to have arisen in the repeated plantings from his dewberry.

Crane (1940*a*, 1940*b*) has resynthesized the loganberry by crossing a wild strain of the octoploid *R. ursinus* (called *vitifolius*), from Berkeley, California, with an autotetraploid form of the European raspberry. In this raspberry the chromosomes had a tendency to associate in groups of 4. Ten seedlings, which were fair matches for the cultivated loganberry, arose from this cross. They, like it, had 42 somatic chromosomes that associated in 21 pairs (Thomas, 1940) despite the cytological irregularities in both of the parents. They, too, were fertile and constant, for they produced 45 F_2 plants similar to the F_1.

The chromosome pairing in this and other hybrids studied by Thomas indicates that the 28 *ursinus* and 14 *idaeus* chromosomes were homologous among themselves. Accordingly, when crossed, these essentially autoploid forms could produce the amphiploid directly. Both the spontaneous and the resynthesized loganberry segregate slightly (Crane and Darlington, 1927; Crane, 1940*b*), but the amount of segregation does not exceed that found in wild species, and the characters distinguishing the parental species do not segregate.

The loganberry appears to have arisen independently in a third instance as the Phenomenal in Luther Burbank's cultures. This form arose as the second-generation offspring of the Cuthbert raspberry and the California dewberry, and it, too, has 21 pairs of chromosomes (Crane, 1935). The Phenomenal is a berry very similar to the logan, with which it has been unable to compete successfully in the trade.

It has not been proved that the raspberry chromosomes are nonhomologous with those of the California dewberry, but in the cross *ursinus* × diploid *idaeus*, 5 to 7 chromosomes remain univalent in the F_1, and these are presumably *idaeus* chromosomes. Also, the systematic remoteness of the parents makes it most unlikely that the genome of *idaeus* would be homologous with one of those from *ursinus*. If they are not homologous, the loganberry would be classified in the lower right-hand corner of table 12.

The loganberry is a very important species commercially, especially from British Columbia to southern California, and it is also cultivated successfully in western and northern Europe. Its forms are very useful in breeding new horticultural races, because they cross so readily with other forms of *Rubus*. In addition to backcrossing with their parents, they will hybridize with other members of the raspberry section, and even with the 7-chromosome *R. parviflorus* Nutt., of the section *Anoplobatus* (C. G. Fraser, 1921, cited in Bailey, 1923).

One of the most successful hybrids derived from the loganberry as one parent is the youngberry (Darrow, 1937), which has the same chromosome number as the logan (Darrow and Longley, 1933). It was produced by crossing the Phenomenal (Burbank's loganberry) to an eastern dewberry, so that presumably some of the *ursinus* chromosomes have been replaced by eastern dewberry chromosomes. The logan and youngberry are intersterile, despite the fact that they have the same chromosome number.

Another possibly related form is the boysenberry that originated in California (Bailey, 1941), for it resembles the loganberry in many characters, although it will probably be impossible to trace its parentage. The laxtonberry (Crane and Darlington, 1927) has received one extra *idaeus* genome in addition to the normal complement in the loganberry, and therefore has $2n = 49$ chromosomes. It is decidedly less fertile than the loganberry.

Rubus titanus Bailey, the Mammoth blackberry, is another true-breeding species that came from Judge Logan's cultures. It arose as a cross between the same form of *R. ursinus* with an eastern blackberry, the so-called Texas Early (Bailey, 1923). From what we now know of the eastern blackberries, this form was probably diploid like the raspberry. The Mammoth blackberry has the same chromosome number as the loganberry (Darrow and Longley, 1933), and has therefore probably arisen in a similar manner, except that it received 14 blackberry instead of 14 raspberry chromosomes. The hybrid between *titanus* and *loganobaccus* is sterile, even though the parents have 28 *ursinus* chromosomes in common. Darrow and Longley observed poor pairing of chromosomes in this hybrid, possibly indicating lack of homology between the 7 blackberry chromosomes from *titanus* and the 7 raspberry chromosomes from *loganobaccus* (Thomas, 1940). This is a fair indication that blackberry and raspberry chromosomes are nonhomologous.

Apart from *Rubus titanus*, the four or five recorded experimental amphiploids each combine the genomes of species belonging to the sections *Idaeobatus* and *Eubatus*. It is apparent from the experimental investigations that *Rubus* corresponds to one immense comparium, and that its sections or subgenera probably correspond to cenospecies.

The thirty-one cases of ploidy discussed in detail above are taken from several of the largest families of seed-bearing plants, with one example each in the Ranunculaceae, Malvaceae, Primulaceae, Labiatae, and Scrophulariaceae, three in the Cruciferae, five in the Solanaceae, and six each in the Gramineae, Rosaceae, and Compositae. From this it appears likely that any trends observed are of a general nature.

The cases reviewed are seen not to be distributed evenly through the boxes of table 12 (p. 72). This unevenness would have been still more obvious if this review had systematically included autoploidy as well as amphiploidy, for then the cases would more clearly be seen to cluster in the upper left-hand and the lower right-hand corner boxes, representing clear examples of autoploidy and amphiploidy, respectively. The important point is that when polyploids are classified according to their breeding behavior and the relationship of their parents, the majority fall into these two classes. In the remaining boxes there are scattered cases of intermediate character. The segregating and most unsuccessful amphiploids are found in the upper half of the table, and the most constant ones in the lower half.

CHARACTERIZATION OF AUTOPLOIDY AND AMPHIPLOIDY

At present there is confusion in the literature regarding the definition of different kinds of ploidy. This is largely due to advances in knowledge that have resulted in changing concepts. When Kihara and Ono (1926) first proposed the concepts of auto- and allopolyploidy and defined these terms, hybrid fertility and chromosome homology were thought to be strictly correlated. Consequently, their definitions do not take care of such polyploids as arise from sterile interspecific hybrids with paired chromosomes. Darlington (1932) modified these concepts by placing the emphasis on chromosome homology as expressed by pairing after doubling, a practice followed by Müntzing (1936*b*). If this criterion is employed, however, *Primula kewensis* and *Aquilegia Janczewskii*, which arose from undoubted interspecific hybrids, would be regarded as autoploids, whereas experimentally produced autoploids with chromosomes conjugating as bivalents rather than as tetravalents would have to be classed as alloploids. The prevalent confusion in the definition of these terms is graphically illustrated in a table by Fagerlind (1937, p. 395).

The present writers propose to limit *autoploidy* to the multiplication of genomes within the limits of one ecospecies. By this definition, autoploidy applies to cases ranging from the homozygous individual with multiplied chromosome number, at one extreme, to the polyploid derivatives of a hybrid between subspecies or ecotypes of a species, at the other.

Since the genic balance relations are the same within the limits of the ecospecies, this definition appears to be both logical and in close agreement with the original concept of the term. Autoploids will always be truly polyploid, for one can scarcely conceive of two natural forms with different numbers of chromosomes being able to interchange their genes freely. Autoploids of hybrid origin will segregate, but the segregants will not be weaker than the parental forms. The greatest handicap of autoploids is that they have more than two chromosomes of a kind, for these multiple homologies will frequently cause irregular distribution of chromosomes to the sex cells, whereby minor aberrations in chromosome number will arise and the genetic balance will be disturbed.

Randolph (1941), in discussing cases of autoploidy, emphasizes the point that there is a variation in the amount of fertility in different autoploid stocks, and that many are quite sterile, but that it should be possible by selection to increase fertility. Even though complete fertility is not regained, the increased seed size, increased nutritional value, and improved growth habit, etc., of autoploids may more than compensate for a partial reduction in fertility.

Amphiploidy, in contrast with autoploidy, is the addition of all the chromosomes of distinct species. In the strictest sense of the term amphiploidy, these species belong to distinct cenospecies. In the wider sense, amphiploidy would also include the addition of the chromosomes of ecospecies of one cenospecies, but these cases are transitional in character and generally less stable.

If the basic diploid parents have the same chromosome number, amphiploids following an arithmetical progression will result from the addition of their genomes. This type of seriation, called polyploidy, characterizes many groups of plants (Winge, 1917). For example, *Nicotiana silvestris*, *tomentosiformis*, and *glutinosa*, all with 12 pairs of chromosomes, gave rise to synthetic *Tabacum* and *digluta*, with 24 and 36 pairs, respectively. If the parents have dysploid numbers, their amphiploids will also be dysploid (and these cannot rightly be termed alloploids). This situation is exemplified by the *Brassica* comparium, where the species of the diploid level, *nigra*, *oleracea*, and *campestris*, have $n = 8$, 9, and 10, respectively, and their possible amphiploid derivatives, *carinata*, *juncea*, and *Napus*, have $n = 17$, 18, and 19. Thus, both arithmetical and dysploid series of chromosome numbers may arise by amphiploidy. Autoploidy, on the other hand, gives rise only to geometrical progressions, as for example in *Eragrostis Cambessediana*, *albida*, and *pallescens*, with $n = 10$, 20, and 40 chromosomes (see p. 134).

Requirements for the success of amphiploids were formulated in chapter V. The present examples have shown how these principles operate in different plant groups, and that interchanges between the parental genomes constitute the most serious threat to the success of amphiploids. It is for this reason that the most successful ones arise from parents between which such interchanges are impossible.

Success also depends upon the availability of an environment in which the new form may become established. In this respect the amphiploid may have an advantage over the autoploid. Its bispecific nature, often combining the genomes of plants fitted to distinctly different environments, enables it normally to fit an environment somewhat different from that occupied by either parent, and to have a greater latitude of tolerance than either parent alone.

METHOD OF ORIGIN OF AMPHIPLOIDS

The manner in which amphiploids arise has aroused much discussion. Naturally it should, since the exact origin of only a few examples has been safely established by experiment. The relative significance of somatic doubling in the F_1 as compared with gametic doubling has been considered by Jörgensen (1928) and Winge (1932). Without doubt both methods of chromosome duplication take place, and sometimes multiplication by autoploidy may precede the hybridization by which the amphiploid finally arises, as appears to have been the case in *Zauschneria*, *Galium*, and *Tradescantia* (see chapters VII and VIII).

Of the eighteen instances cited in table 12, twelve arose through gametic doubling, four through somatic doubling, and two through both types. It is probably significant that three of the cases of somatic doubling involved hybrids with completely homologous chromosomes, namely, the cases in *Fragaria*, *Primula*, and *Aquilegia*. This means that the amphiploids in the strictest sense have almost all arisen from the unreduced gametes of a sterile hybrid.

The circumstances under which amphiploids arise should be considered in practical breeding work, both with respect to the degree of relationship between the parents and the method of origin. In most of the results obtained with colchicine, the chromosome duplication is somatic. The natural gametic elimination is thus temporarily avoided, but it has to be faced later. Consequently, many somatically doubled hybrids are likely to prove unsuccessful after a few generations. If the amphiploids have means for vegetative reproduction by apomixis or otherwise, only the initial balance of the F_1 hybrid is important. For such groups of plants somatic doubling not only would prove suitable, but might also be the efficient means of attaining the desired result.

Amphiploids may come into existence well balanced and fully fertile from their inception, but some require two or more generations in which to accomplish these ends. During this period slight readjustments between the parental genomes may take place. This appears to have occurred in synthetic *Galeopsis Tetrahit*, which is thought to have received at least one partially recombined genome, and in the fertile *Nicotiana Tabacum* obtained by Kostoff, as contrasted with the female-sterile form produced by Greenleaf and R. E. Clausen following somatic doubling. In the former tobacco, off-balance gametes could be eliminated; in the latter, there was no such opportunity. If this kind of elimination is drastic, it is an effective mechanism for promptly selecting an amphiploid with the constitution required for success. It would doubtless account for the fact that most of the successful amphiploids arose through the gametic process.

When the right balance is at hand, the amphiploid may appear. Conditions may favor its reappearance several times, as is shown by the repeated production of *Madia nutrammii*, *M. citrigracilis*, and *Triticale*, and by the synthesis of the wild amphiploid species *Phleum pratense*, *Galeopsis Tetrahit*, *Brassica Napus*, and *Nicotiana Tabacum*. Even when a roundabout procedure is followed, as in the production of *Nicotiana Tabacum*, *Galeopsis Tetrahit*, and *Phleum pratense*, the genic balances predetermined by the constitution of the parental genomes are usually obtained successfully.

Diploid gametes have been observed by the writers in twelve different interspecific hybrids in *Layia* and *Madia*. Nine of these were inter-cenospecific hybrids; all followed the semi-heterotypic pattern in their meiotic divisions, but only the three reported in chapters II, III, and IV established true-breeding amphiploids, although five others had equal opportunity to do so. The other three hybrids were intra-cenospecific. These observations indicate that even though nondisjunction of unpaired chromosomes and diploid gametes may be fairly common phenomena in inter-cenospecific hybrids, their presence does not guarantee that an amphiploid will result.

VII

ECOLOGIC CHARACTERISTICS OF NATURAL AMPHIPLOIDS

Two fundamental factors governing polyploids have been emphasized in the preceding chapters. The first of these is the cytogenetic mechanisms that operate in their production, and the second is the relationships between their progenitors. A third factor of equal significance remains to be considered. This is the ecological behavior of natural amphiploids as compared with that of their progenitors, which is the topic of the present chapter; and that of natural autoploids, which forms the subject matter of the following chapter. Several of the better-known cases on record furnish the necessary data.

Not many natural amphiploid species have been artificially synthesized, and so the acceptance of additional species as being of amphiploid origin rests largely upon circumstantial evidence. This is principally of four kinds, namely, cytological, genetical, morphological, and geographical. Data from these sources combine to yield a general pattern of evidence by which amphiploids may be recognized with some degree of certainty. Genetic data are frequently lacking because of the extensive investigations required, but where they have been obtained, the case becomes less circumstantial and more nearly proved.

The following review of examples will illustrate the general relations between the ecological position of natural amphiploids and that of their probable parents. The known cases which have been fairly carefully studied are relatively few. The following survey is not exhaustive, but represents diverse genera and probably gives a fair over-all impression of the relative significance of amphiploids in the ever changing natural environments.

1. *Spartina Townsendii* H. et J. Groves, $n = 63$. This is probably the only amphiploid which is known to have arisen spontaneously in historic times. This cord grass appeared in the English Channel around 1870 and was supposed to be a hybrid between the native *S. stricta* (Ait.) Roth and the introduced North American species, *S. alterniflora* Lois. Not only does it combine several of the distinctive morphological and anatomical characters of the supposed parental species, but it was found in the one northern European locality where the two have met. *Spartina Townsendii* did not spread very rapidly for the first thirty years or so

of its existence, but it has now expanded widely from its point of origin and has almost completely eliminated its parent species wherever it has come in contact with them. Huskins (1931) determined the chromosome numbers and found 28 pairs in *stricta*, 35 pairs in *alterniflora*, and 63 pairs in the new species, *Townsendii*, the latter number being the sum of the others. Both the historical and the cytological information provide strong circumstantial evidence that *Townsendii* evolved through the addition of the chromosomes of *stricta* and *alterniflora*. This very successful new species has attained considerable economic importance as a mud-binder along the west coast of Europe.

2. *Phleum pratense* L., $n = 21$. The artificial synthesis of the cultivated timothy by Gregor has been reviewed in the preceding chapter. The diploid parent, *P. nodosum* L. ($n = 7$), is a species of dry, uncultivated situations in the lowlands, whereas the other parent, *P. alpinum* L. ($n = 14$), occurs in moist situations at high altitudes or latitudes. Their amphiploid, *P. pratense*, is exceedingly adaptable over a wide range of environments, but thrives best in intermediate, lowland situations. This is clearly a case in which the amphiploid combines the capacities of the parents to survive in different kinds of habitats, and is therefore adapted to occupy a greater range of environments than they.

3. *Poa annua* L., $n = 14$. The annual bluegrasses are contained in the section *Ochlopoa* of the genus *Poa*. Their most widely distributed member is *Poa annua* L., the type species of the section. From Nannfeldt's investigations (1937) on the cytology and distribution of this species and of its relatives in Europe, it appears likely that *annua* originated as an amphiploid.

There are two 7-chromosome members of the section *Ochlopoa* in Europe. One is *Poa exilis* (Tomm.) Murb., which is a tender Mediterranean, strictly annual species. The other is *Poa supina* Schrad., a subalpine-temperate perennial of moister, cooler habitats, which extends above 3000 meters in the Alps and northward to central Scandinavia. They meet in the mountains of the Mediterranean region, and have been usually treated as varieties or subspecies of *Poa annua*.

In certain characters the tetraploid *Poa annua* is like one diploid or the other; in others it is intermediate between them, as is shown by Nannfeldt in a tabulation. It is an ephemeral annual or a perennial, but has a much larger ecologic amplitude than the diploids, for it is truly cosmopolitan and extends from the Arctic to the tropics and from low to high altitudes. Nannfeldt tried to cross the two diploid species, but was unable to obtain a hybrid from the strains he used. Hybrids between *annua* and *supina* are fairly frequent in natural habitats, however, and he showed these to be triploids.

The crossing experiments are not extensive enough to be conclusive, but they suggest that *Poa exilis* and *P. supina* are genetically more distantly related than their morphological characters would indicate. Also, the occurrence of hybrids between the tetraploid and one of the diploid species is a pattern characteristic of many amphiploids. The section *Ochlopoa* is very isolated in the genus, and these three species have no other close relatives in Europe. For this reason, the morphological, cytological, and ecological evidence is very significant and suggestive of an amphiploid origin for *Poa annua*. This species is exceedingly successful and has been able to follow man around the world.

4. *Iris versicolor* L., $n = 54$. This is a well authenticated example of the manner in which an apparent amphiploid occurs between its supposed parents (Anderson, 1936). This species is geographically wedged between *I. setosa* Pall., $n = 19$, with a relict distribution around the Gulf of St. Lawrence, and *I. virginica* L., $n = 35$, of the Mississippi Valley and the southeastern United States. *Iris versicolor* is found in the northeastern corner of the United States left unoccupied by the other two, and in addition covers the entire relict distribution of *setosa* to the north, and the borders of the area of *virginica* to the south and west. This overlapping is possible because the three species have different habitat preferences. The southern species, *virginica*, is a plant of the swamps; *setosa* occurs in natural prairies in the north; and the amphiploid *versicolor* grows in the intermediate zone between the wet lowlands and the dry talus.

Extensive statistical measurements show that the morphological characters of *versicolor* are intermediate between those of the other two, but that it is closer to *virginica*, which has approximately twice as many chromosomes as *setosa*. Anderson has presented the theory that in preglacial or interglacial times *versicolor* arose in the Great Lakes region by amphiploidy from hybrids between *virginica* and some form of *setosa* which is now extinct there. A form of *setosa*, fitting the morphological requirements of a progenitor of *versicolor*, occurs in the interior of Alaska in the region that escaped Pleistocene glaciation. Anderson suggests that populations of *setosa* once covered much of Canada, and that the hypothetical form of the Great Lakes region which he suspects gave rise to *versicolor* may have been similar in appearance to that now found in interior Alaska.

5. *Eriogonum fasciculatum* Benth. ssp. *foliolosum* (Nutt.) Stokes, $n = 40$. This presumed amphiploid provides another example of a derived polyploid occupying an area between the ranges of its progenitors (Stebbins, 1942). *Eriogonum fasciculatum*, the common shrubby wild buckwheat of the southern half of California, in the broad sense as usually treated by taxonomists constitutes a polyploid complex that evidently consists of two or more distinct ecospecies. Subspecies *typicum* Stokes, $n = 20$, is native in the coastal region of southern California, whereas ssp. *polifolium* (Benth.) Stokes, $n = 20$, is interior and borders the California deserts. Between them occurs an intermediate group of forms, ssp. *foliolosum*, with $n = 40$. The map, figure 2 in Stebbins' paper, illustrates the geographic-ecologic relations of the suspected amphiploid, *foliolosum*, with *typicum* and *polifolium*. The morphological differences between these three units are not so great as those often found between species of *Eriogonum*, and at present there are not sufficient data available to determine whether or not *typicum* and *polifolium* are intersterile, and hence distinct species. If they are but ectoypes of one species, *foliolosum* should be classified as an autoploid, which would indeed be unique if it arose from an ecotype cross. But in that case it should segregate forms fitted to occupy the climates of its putative parents and spread to their areas, and this it has not done, although it overlaps with them to some extent.

6. *Rumex Acetosella* L., $n = 21$ (Löve, 1941). In this preliminary report, Löve presents data on the cytology and systematics of the European members of the subgenus *Acetosella*, and maps their distributions. He finds that this Linnaean species is a complex, with chromosome numbers in a polyploid series from $n = 7$ to $n = 28$.

In the warm areas of southwestern Europe and the Mediterranean region, there is a 7-paired species, *Rumex angiocarpus* Murb., and in northeastern Europe, including the Arctic, is a 14-paired species, *R. tenuifolius* (Wallr.) Löve. These two have somewhat overlapping distributions. A hexaploid, *R. Acetosella* L., $n = 21$, is found with the tetraploid, although on slightly different soils. It does not extend as far north, but extends farther south. Finally, an octoploid species, *R. graminifolius* Lamb., $n = 28$, is distributed from the Kola peninsula in Russia to Alaska. The distributional pattern of the diploid, tetraploid, and hexaploid sheep sorrels is rather parallel to that found in the *Poa annua* group reviewed above.

Morphologically, *Rumex angiocarpus* differs from the others in that the large inner calyx is attached to the fruit, and in having broad, short fruits and seeds. Its growth habit is erect, and its leaves are broadly hastate. *Rumex tenuifolius* and *R. graminifolius* have very narrow leaves, a decumbent habit, and more elongate seeds. *Rumex Acetosella* resembles *angiocarpus* in its habit and leaves, but *tenuifolius* in its fruit characters. These resemblances, together with the chromosome numbers and the lack of close relatives, strongly suggest that *Acetosella* may have arisen as an amphiploid of *angiocarpus* and *tenuifolius*. Because *graminifolius* is limited to the Arctic, resembles *tenuifolius*, and has twice as many chromosomes, Löve suggests that it may have originated through autoploidy.

The sheep sorrels have followed man and are almost cosmopolitan in distribution. Because *Acetosella* and *graminifolius* have been hitherto the only generally recognized species of this complex, it is not known whether the diploid and the tetraploid species occur outside the Old World. *Rumex tenuifolius* appears not to have reached North America, for the forms of the eastern United States and Canada, as well as those from northern California to Alaska, are morphologically similar to the hexaploid, *Acetosella*. This observation has received some cytological verification from Jensen (1936), who found that plants from Nova Scotia and Massachusetts were hexaploid.

Amphiploidy and autoploidy seem to account for the origin of the two species with the highest number of chromosomes in this complex, but the supposedly amphiploid *R. Acetosella* is evidently the most aggressive of its members.

7. *Zauschneria californica* Presl ssp. *typica* Keck, $n = 30$. This presumably amphiploid form of a small western North American onagraceous genus follows a more complex pattern of origin than the other examples, but the ecological circumstances are similar to those already discussed. The natural units of this genus were discussed in detail, illustrated, and mapped in a previous publication (Clausen, Keck, and Hiesey, 1940). The relations of the California forms, as deduced from cytogenetic evidence, morphological resemblance, and proximity in distribution, are shown by the diagram, figure 85.

Two restricted, diploid endemics are found in the coastal regions of California. One is a narrow-leaved semishrub, *Z. cana* Greene, of the southern coast, and the other is a broad-leaved herbaceous perennial of the cool, moist redwood region of the northern part of the state, *Z. septentrionalis* Keck. These species are separated by a distance of 350 miles, so they have no opportunity for hybridizing naturally.

Each diploid has a tetraploid morphological counterpart, and in geographic distribution these bridge the gap between the diploids. One, Z. *californica* ssp. *angustifolia* Keck, is probably an autoploid of *cana*, for it resembles it closely, follows it in distribution, and shows similar reactions when transplanted. The tetraploid counterpart of *septentrionalis* is Z. *californica* ssp. *latifolia* (Hook.) Keck. Unlike the restricted diploid, this form is widespread through the mountains from southwestern Oregon to New Mexico and is variable in form. In the North Coast Range of California it occurs near the area occupied by *septentrionalis* (from which it may have arisen through autoploidy), and in the mountains of southern California it approaches the zone occupied by ssp. *angustifolia*.

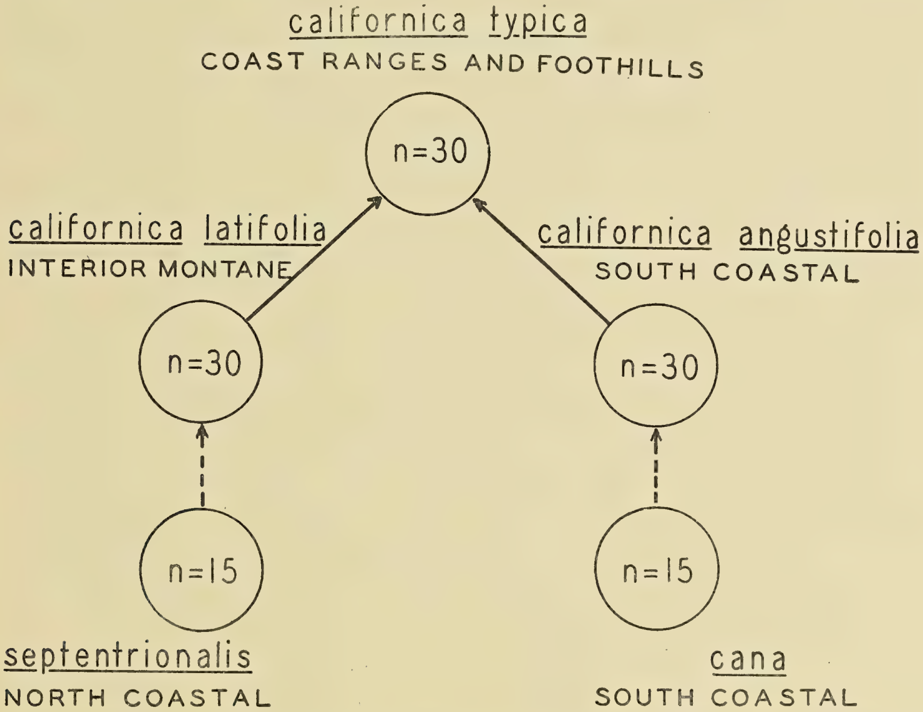

FIG. 85. Relationships of the California forms of *Zauschneria*. Broken lines indicate hypothetical formation of autoploids; solid lines, steps verified by hybridization.

The suspected amphiploid, Z. *californica typica*, is found in the area that is left between the others, principally through the Coast Ranges of central and southern California. This form is morphologically intermediate between ssp. *latifolia* and ssp. *angustifolia*, although quite variable, and when transplanted to different altitudes it reacts in a manner intermediate to these two subspecies (Clausen, Keck, and Hiesey, 1940, figs. 88, 97).

The hybrid origin of *Z. californica typica* is strongly suggested by the fact that F_1 hybrids between the autoploids, sspp. *angustifolia* and *latifolia*, duplicate it in appearance. These hybrids are fully fertile, but in large F_2 populations of more than 3000 individuals they segregate only counterparts of the variable *californica typica*, and no parental types. This must mean that there is preferential pairing between the two sets of 15-chromosome genomes from each parent. The morphological, cytological, ecological, and experimental facts have been interpreted to mean that *californica typica* is in a sense a delayed amphiploid of the two diploid species, *cana* and *septentrionalis*, produced by way of the autoploids, *californica* sspp. *angustifolia* and *latifolia*. In *Zauschneria*, therefore, amphiploidy has been superimposed upon autoploidy in a manner similar to that observed in *Tradescantia* and *Galium*, which are reviewed in the following chapter. Nevertheless, the amphiploid derivative is found in an environment different from and somewhat intermediate between those of its putative diploid ancestors.

8. *Galeopsis Tetrahit* L., $n = 16$. This natural Linnaean species has been successfully synthesized by Müntzing, as reviewed in some detail in chapter VI. Both of the diploid parent species, with 8 pairs of chromosomes, are nearly limited in distribution to central and eastern Europe, where *G. pubescens* Bess. is a lowland and foothill ecospecies, and *G. speciosa* Mill. is montane, being even subalpine and subarctic. Their amphiploid, *G. Tetrahit*, has a far wider distribution than either, extending from the Faeroe Islands to Siberia, Japan, and northwest India, over even higher mountains than *speciosa*.

9. *Penstemon neotericus* Keck, $n = 32$ (Keck, 1932; J. Clausen, 1933). This endemic species of the northern Sierra Nevada in California occupies an area skirted by its supposed parents, *P. laetus* A. Gray, $n = 8$, and *P. azureus* Benth., $n = 24$. A map showing the distribution of these species and a description of the morphological and cytological differences between them are given in Clausen's 1933 paper. The inflorescence of *neotericus* resembles that of *laetus*, but the remainder of the vegetative body is like that of *azureus*. The ecotype of *laetus* that most likely is the parent of the amphiploid is a foothill form associated with *Pinus Sabiniana*, and the form of *azureus* is native at higher levels and extends up through the Canadian zone, where it is an associate of *Pinus Jeffreyi*. The octoploid *neotericus* occupies an intermediate belt in association with *Pinus ponderosa* in the Transition zone. In this case the amphiploid is of more limited distribution than either of its supposed parents.

10. *Madia citrigracilis* Keck, $n = 24$. A full account of this amphiploid is given in chapter III. Like *Penstemon neotericus*, cited above, *Madia citrigracilis* occurs between its parents, *M. gracilis* (Sm.) Keck, $n = 16$, and *M. citriodora* Greene, $n = 8$, and its distribution appears to be limited to a restricted area. Its present endemism may mean either that this is a very young species, or that it is not sufficiently aggressive to expand its range in the face of competition from other plants with similar ecological requirements.

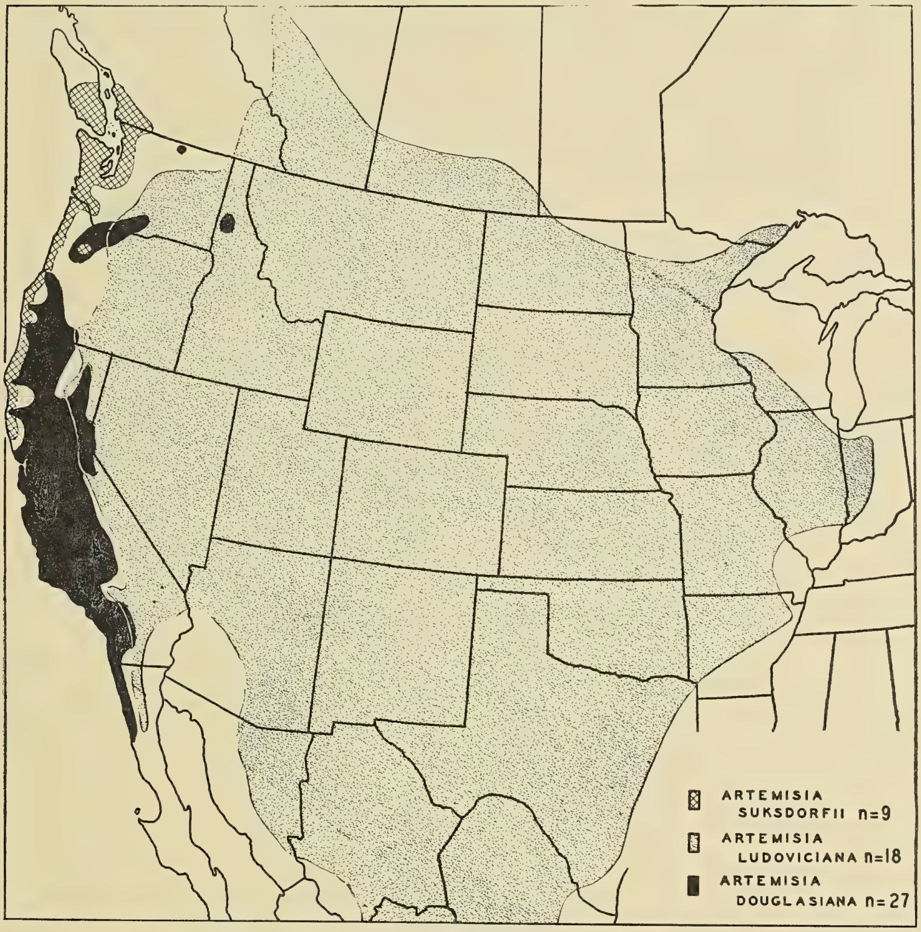
▨ ARTEMISIA SUKSDORFII n=9
░ ARTEMISIA LUDOVICIANA n=18
■ ARTEMISIA DOUGLASIANA n=27

Fig. 86. Distribution of *Artemisia Douglasiana* and its presumed parents

11. *Artemisia Douglasiana* Bess., $n = 27$. This species apparently originated through the addition of the chromosomes of *A. Suksdorfii* Piper, $n = 9$, and *A. ludoviciana* Nutt., $n = 18$ (Clausen, Keck, and Hiesey, 1940; Keck, in press). The distribution of these three species is shown on the map, figure 86.

The supposed diploid progenitor, *A. Suksdorfii*, is a distinctly coastal species, occupying a narrow zone from southern British Columbia to central California. The putative tetraploid parent, *A. ludoviciana*, is very widely distributed through the interior regions of North America. It has developed many ecotypes and occurs from the Cascade–Sierra Nevadan chain eastward to the Great Lakes. In the Columbia River gap it approaches the range of *Suksdorfii*.

The hexaploid species, *A. Douglasiana*, grows in those regions which lie between the areas of its probable parents, and the ranges overlap but little, as is indicated in the map. It is intermediate between the other two forms in its reaction to transplanting to very unlike environments, as was reported in 1940, and it is likewise morphologically intermediate between them, as discussed in both papers cited, and as illustrated in figures 128 and 129 of the 1940 paper. Consequently, its morphology, number of chromosomes, ecological characteristics as revealed by transplant experiments, and geographical distribution combine to produce strong circumstantial evidence for its amphiploid nature.

The examples presented, though far from exhaustive, suggest the general trends to be found in the ecological relations between amphiploids and their parents. The amphiploids usually occur in intermediate environments. In some cases, for example *Penstemon neotericus* and *Madia citrigracilis*, they appear to be able to establish themselves only as local endemics, and possibly never will be able to extend their range, or successfully invade those of their parental species. In others, for example *Artemisia Douglasiana*, *Eriogonum fasciculatum* ssp. *foliolosum*, and *Iris versicolor*, they are quite widespread, but still chiefly limited to intermediate environments. In yet others, for example *Galeopsis Tetrahit*, *Phleum pratense*, *Spartina Townsendii*, *Poa annua*, and *Rumex Acetosella*, they appear able to grow in the habitat of either parent as well as in intermediate surroundings, so that their distribution becomes very extensive and the species are outstandingly successful.

The success of amphiploids in the wild depends upon the interplay between their new genetic combination and the available environment. Therefore, they may be fitted to occupy a wider range of habitats than either parent alone, or the reverse may be true. Their degree of fitness measures their capacity to withstand both the physical factors of their environment and close competition with other plants. Those amphiploids that have succeeded must have been at least as able to withstand natural selection as their parents, but it is also obvious that they greatly differ in their capacities in this regard.

Hybrids between ecotypes fitted to very different environments often have a wider range of ecological tolerance than their parents, but such hybrids will segregate forms in later generations adapted variously to the environments. Also, as mentioned above, amphiploids that combine the genomes of species native to different climates may have a greater ecological amplitude than their parents. If the chromosomes of the parental species are unable to pair in such amphiploids, the wider ecological tolerance can be maintained; but if there is intergenomal pairing, segregation may be expected for this character. These principles are probably of importance in plant breeding.

VIII

ECOLOGIC CHARACTERISTICS OF NATURAL AUTOPLOIDS

Relatively few natural polyploids reported in the literature can be regarded as clear examples of autoploidy. This is true largely because various criteria have been used in judging autoploids, and also because the investigations have been too incomplete to permit an accurate evaluation of the kind of ploidy reported. The following examples will make this clearer.

Penstemon azureus ssp. *parvulus* (A. Gray) Keck, $n = 16$, and *P. azureus* ssp. *angustissimus* (A. Gray) Keck, $n = 24$, were listed as autoploids by Müntzing (1936*b*, p. 272) because one or two quadrivalents are common in the 24-chromosome form. The criterion of multivalent association as an indicator of autoploidy has since been found invalid, because it is now known that in completely sterile interspecific F_1 hybrids the chromosomes may be completely homologous. *Penstemon azureus* belongs to a polyploid complex whose members are morphologically very closely related. Subspecies *angustissimus*, instead of being an example of autoploidy, is even more plausibly an amphiploid derivative of ssp. *parvulus*, $n = 16$, and *P. heterophyllus* Lindl., $n = 8$.

If an amphiploid arises from hybrids between species whose chromosomes are homologous, some multivalent association may be expected, as has been observed in *Primula kewensis*. Also, not all autotetraploids have quadrivalent chromosomes. An especially interesting case was reported by Skirm (1942), who produced autotetraploids in *Tradescantia* by thermal shock, and found that although some of them had quadrivalents, a sister plant had bivalents only.

Another example is the *Madia gracilis* complex discussed in chapter III. This consists of *M. subspicata*, $n = 8$, tetraploid *gracilis*, $n = 16$, hexaploid *gracilis*, $n = 24$, and *citrigracilis*, $n = 24$, in addition to the forms that cluster around *M. sativa*, $n = 16$. These are morphologically so closely related that investigators unfamiliar with the entire genus and its patterns of inheritance would scarcely hesitate to regard them as examples of autoploidy unless they were cautioned by the additive chromosomal series, $n = 8$, 16, and 24. The synthesis of hexaploid *citrigracilis* from a hybrid between tetraploid *gracilis* and diploid *citriodora* proved that amphiploidy could take place in this complex. Moreover, the almost complete lack of pairing between the 24 chromosomes of the *gracilis* ×

citriodora F_1 hybrid indicates that the 16 chromosomes of *gracilis* are nonhomologous not only with those of *citriodora,* but also inter se, contrary to expectation if tetraploid *gracilis* were an autoploid derivative of *subspicata.* Species complexes, therefore, cannot be considered to contain autoploid series on the basis of morphology and chromosome number and pairing in nonhybrids alone.

Included in Müntzing's list of autoploids are also several instances of forms with dysploid chromosome numbers. In these cases the dysploid forms had been considered conspecific, which was obviously Müntzing's reason for including them. But dysploidy cannot arise through autoploidy, nor through chromosome doubling in a hybrid between forms with the same chromosome number. Forms that produce new dysploids invariably belong to distinct species. For example, Müntzing includes *Erophila verna* (L.) E. Mey., but, as has been shown by Winge (1940), this is in reality a cenospecies containing several ecospecies. Its forms with new chromosome numbers evolved both through amphiploidy and through the segregation of hybrids between species with different, usually dysploid, chromosome numbers.

EXAMPLES OF NATURAL AUTOPLOIDS

In the following pages some examples of probable autoploids are reviewed in order to evaluate their ecologic and evolutionary significance as compared with amphiploids. Fairly safe examples of true autoploids can be recognized only in essentially monotypic genera and sections, and in those groups that have been thoroughly investigated cytogenetically.

1. *Galax aphylla* L., $n = 6$ and 12 (Baldwin, 1941). This is one of the simplest and most clear-cut cases to be found, for the genus *Galax* of the family Diapensiaceae, which contains few genera and species, has only this one morphological species. *Galax aphylla* occurs from Chesapeake Bay to central Alabama, principally in the Appalachians but also on the coastal plain. As is shown by Baldwin's investigation of several hundred plants from a total of 117 stations, the diploid and tetraploid forms grow intermixed throughout the entire range of the species, and morphologically they are very similar except that under the most favorable growing conditions the tetraploid is the larger. No multivalent chromosome associations were detected in meiosis of the tetraploid. Since neither ecologic, geographic, nor morphologic barriers were found to separate these chromosomal races, they do not qualify as taxonomic species, although they are probably isolated genetically, in which case

they would be species in the genetic sense. The relation in form and distribution between this autoploid and its parent contrasts sharply with the situations found in the amphiploids reviewed in the preceding chapter.

2. *Empetrum nigrum* L., $n = 13$, and *E. hermaphroditum* (Lge.) Hagerup, $n = 26$ (Hagerup, 1927). The black crowberry furnishes one of the early spectacular examples of polyploidy within what has been considered to be one species, and for that reason has been widely cited. The Empetraceae as a family appears to be depleted, with no close relatives. Even its position in the natural system is still open to much doubt. It consists of three genera: *Ceratiola*, a monotypic endemic of the southeastern United States; *Corema*, the brown crowberry, with two species, one on either side of the North Atlantic; and *Empetrum*, with the circumpolar *nigrum* complex in the northern hemisphere, and *E. rubrum* Vahl ex Willd. in the southern, from the southern tip of South America, the Falkland Islands, and Tristan da Cunha. The northern and southern species of *Empetrum* are so closely related morphologically and ecologically that some authors have considered them to be only one species despite their peculiar geographical separation.

Empetrum nigrum in the broad sense is a complex consisting of small, ericoid bushes found on heaths, moors, dunes, and mountainsides. It is dioecious in most parts of the Temperate Zone, with male and female flowers on different plants. In the Arctic, on the other hand, it is monoecious, with male and female flowers on the same plant. The sexual characters of the *nigrum* complex are fairly modifiable, however, and sometimes both sexes will develop in one flower. Consequently, taxonomists disregarded the bisexual form (distinguished as forma *hermaphroditum* by Lange in 1880) until Hagerup pointed out that it differed from the dioecious both ecologically and cytologically.

The bisexual crowberry of the Arctic and Subarctic and of high altitudes farther south is a matted, heavily fruiting form with 26 pairs of chromosomes. The dioecious form from lower altitudes in the Temperate Zone, on the other hand, is more ascending, with flowers and fruit more scattered, and with only 13 pairs of chromosomes. The two forms occur together over a wide area, as for example in central Sweden and in the Faeroe Islands. The exact boundaries of the two have not been mapped, but Hagerup indicates that *hermaphroditum* is the only form in Greenland, and Polunin (1940) recognizes only this form in eastern arctic Canada. The diploid, dioecious form is the only one found in Denmark and in Sweden south of the lake region (Arwidsson, 1935).

There is no doubt that two ecospecies are involved, namely, *Empetrum nigrum*, the diploid of the northern Temperate Zone, and *E. hermaphroditum*, the tetraploid of arctic and alpine regions. The distributional pattern in the genus and family appears to exclude amphiploidy as the means of origin of *hermaphroditum*. The morphological similarity between this species and *nigrum*, and the fact that a fairly high prime chromosome number has been doubled, favor the explanation of its origin by autoploidy.

Unlike tetraploid *Galax*, the tetraploid *Empetrum* inhabits a different environment from its diploid counterpart, and this separation has been accompanied by some morphological differentiation.

3. *Vaccinium uliginosum* L., $n = 24$, and *V. u.* var. *microphyllum* Lge., $n = 12$ (Hagerup, 1933). The genus *Vaccinium* has a center of diversification in the eastern United States. Only three of its species complexes are circumboreal to circumpolar. These are typified by *V. Vitis-Idaea* L., the cowberry, *V. Myrtillus* L., the bilberry, and *V. uliginosum* L., the bog bilberry. These three very distinct species are the only ones, except *V. Oxycoccus* L., that occur in Europe, and only *Vitis-Idaea* and *uliginosum* occur in the Arctic.

The type form of the bog bilberry, *V. uliginosum*, of northern and central Europe, is a rather large-leaved, erect bush of lowland bogs and moors, but other races are found in montane and arctic regions. In eastern North America only subalpine races of this large-leaved type appear to have survived, whereas in the western United States as far south as southern Oregon it occurs near the coast in a lowland belt, as in Europe.

A smaller-leaved, slenderer form of quite different type occurs in high alpine and arctic regions. This was early recognized under various names, viz., var. *alpinum* Bigel. in arctic Canada, var. *microphyllum* Lge. in northern Greenland, and var. *frigidum* Schur. in the Alps. The northern Greenland type described by Lange has leaves only one-third to one-fourth as long as the typical form of the species. It occurs in dry hills between 60° and 76° north latitude and up to 700 meters elevation; the typical form is also found in Greenland, but south of 64° north latitude in moist places.

Hagerup investigated both forms from Greenland cytologically and found that the more northern one, *microphyllum*, is diploid, with $n = 12$, and the more southern, typical *uliginosum*, is tetraploid, with $n = 24$. In Denmark he found tetraploids only. Specimens similar to the diploid form were noted from arctic North America, Newfoundland, the Shetland Islands, northern Norway, Siberia, and the Alps at 2400 meters.

This suggests that the diploid is circumpolar, and possibly had a wider distribution before the glacial period.

From the morphological and phytogeographical evidence it seems unlikely that tetraploid *uliginosum* could have arisen as an amphiploid from any of the three circumboreal species of *Vaccinium.* Its close similarity to the diploid *microphyllum* suggests that it probably arose as an autoploid from that form.

Biosystematically, *V. uliginosum* and its var. *microphyllum* are good ecospecies. They are separated ecologically both by range and by habitat preference, and genetically by their difference in chromosome number. Their morphological differences suggest that they could be recognized as taxonomic species as well. If it can be shown that the forms from the high Arctic of three continents and from the Alps, mentioned by Hagerup as being similar to the Greenland diploid, are themselves diploid, it would be natural to include all in one arctic-alpine ecospecies.

The *Empetrum nigrum* and the *Vaccinium uliginosum* complexes are cohabitant, but in the former the diploid member is south of the tetraploid, whereas in the latter the reverse is true. A parallel case to the *Vaccinium* has been reported by Böcher (1936) in *Campanula rotundifolia* L. He found in eastern Greenland that a diminutive arctic type is diploid ($n = 17$), and a larger, more subarctic type is tetraploid ($n = 34$). The much larger plants in Denmark are also tetraploid. Böcher, however, does not claim that the tetraploids arose by autoploidy.

The other bilberries and the blueberries of the United States appear to form intricate species complexes, according to the studies of Coville (1927), Longley (1927), and Camp (1942*a*, 1942*b*). The chromosome numbers $n = 12$, 24, and 36 have been found. The situation described by Camp suggests that both auto- and amphiploidy have occurred during the evolution of these complexes, but many experiments will be required to clarify the relationships of these groups. For example, the complete interfertility of many so-called interspecific hybrids suggests that some of the forms recognized as taxonomic species are merely ecotypes.

Among the cranberries of Europe, *Vaccinium Oxycoccus* L. in the broad sense, a parallel situation has come to light through studies of Hagerup (1940), who has discovered races having $n = 12$, 24, and 36 chromosomes. These in part are morphologically and ecologically distinct; for example, the diploid has the northernmost distribution. Hagerup suggests that the hexaploid arose by amphiploidy from the other two.

4. *Eragrostis Cambessediana* (Kunth) Steud., $n = 10$, *E. albida* Hitchc., $n = 20$, and *E. pallescens* Hitchc., $n = 40$ (Hagerup, 1932).

This series of grasses from the vicinity of Timbuktu in French West Africa is a spectacular example of probable autoploidy. It is of special interest that only one species was recognized previously in this group, but that from Hagerup's material Hitchcock admitted three species that coincided exactly with Hagerup's cytological and ecological findings.

The ecological preferences of the three species are vividly described in Hagerup's paper. The diploid *Cambessediana* is an annual that grows in silt beside small lakes, but dies when the yearly inundations reach it. Away from the silt in a narrow zone at the base of near-by dunes the tetraploid *albida* is found. It is similar to the diploid species, but has narrower leaves and the perennial habit. On the very dry dunes themselves, where the sand is often heated to a temperature of 80° C., the octoploid *pallescens* occurs. It is the most robust of the three and obviously perennial, with broad leaves that can inroll and remain functional during the dry period, but otherwise it, too, is similar to *Cambessediana.*

The great morphological similarity between the forms, the definite seriation in their adjacent habitats, and the geometrical progression of their chromosome numbers strongly indicate that these are members of an autoploid series. It should be pointed out, however, that there are two other species of *Eragrostis* in the Timbuktu area (Hagerup, 1930), both of which are annuals with chromosome numbers undetermined.

Since these plants presumably are members of an autoploid series, the question might be raised whether they are not after all chromosomal races of one species. In this instance the answer from the biosystematic viewpoint is that despite autoploidy these should be considered ecospecies of one cenospecies, because their differences in chromosome number would doubtless prevent free interbreeding. Also, they are morphologically distinguishable, and have very different ecologic preferences.

In this *Eragrostis* complex the diploid is found in the moistest habitat, whereas in the *Vaccinium uliginosum* complex the diploid was in drier habitats than the tetraploid. Also, the observed correlation between increase in polyploidy and increase in duration of life may be significant.

All these cases illustrate the fact that autoploidy, in a manner not unlike amphiploidy, may shift the genic balance, thus permitting the plant to invade new environments. It does not, however, appear to force adaptation predominantly toward either severer or milder conditions than those of the place of origin.

5. *Zea mexicana* (Schrad.) R. et M., $n = 10$, and *Z. perennis* (Hitchc.) R. et M., $n = 20$. These two species, commonly known as annual and perennial teosinte, respectively, were referred to the genus

Euchlaena until their recent transfer to *Zea* by Reeves and Mangelsdorf (1942) on the basis of cytogenetic evidence. These were the only two species in the genus *Euchlaena.* This reduction of the two genera to one is in order, as the experimental evidence shows, but one may infer from the following that still further amalgamation is necessary to bring the classification of *Zea* into line with the experimental viewpoint.

Zea mexicana is an annual grass that differs from corn, *Zea Mays* L., in only a few genes which determine striking character differences between the two (Mangelsdorf and Reeves, 1939). It is native in central Mexico, where it is commonly found as a weed of the cornfields, and extends also to Guatemala.

Zea perennis is native to a single locality only one mile square near Guadalajara, Mexico. Its extensive system of rhizomes, which enables it to maintain the perennial habit, and its chromosome number are practically the only differences between it and *mexicana.*

Both teosinte and corn occur as diploids ($n = 10$) and tetraploids ($n = 20$), and they are interfertile on both the diploid (Collins and Kempton, 1920; Arnason, 1936) and tetraploid levels (Emerson and Beadle, 1930; Collins and Longley, 1935). Their chromosomes are very largely homologous, and in their hybrids, which are highly fertile, crossing over between the interspecific chromosomes is about on the same order as that in pure maize. This evidence, in general, suggests that annual teosinte and corn should be biosystematically regarded as members of one ecospecies.

The hybrids between teosinte and corn bring out interesting balance relations between their genomes. When diploid corn is crossed with diploid teosinte, cornlike ears are produced, but in the triploid hybrid between tetraploid teosinte and diploid corn, two-rowed ears occur as in teosinte, indicating the influence of the extra genome of perennial teosinte (Emerson and Beadle, 1930). In the hybrid between tetraploid corn and tetraploid teosinte, however, the extra corn genome restores the balance to that prevalent on the diploid level, for the ears of this hybrid are again cornlike. These facts suggest that the two 10-chromosome genomes of perennial teosinte are of approximately equal value, as one would expect in an autoploid.

There is now conclusive evidence that perennial teosinte is an autoploid of annual teosinte. Randolph (unpublished data, 1943, cited by permission) found among his experimental plants a sectorial chimera of teosinte having both diploid and tetraploid sectors. The diploid sectors were annual, whereas the tetraploid became perennial. He also found, however, other races of *Z. mexicana* which, when artificially doubled by heat treatments, did not produce plants having the perennial habit. This finding is explained on the basis of gene differences among races of *mexicana*, some of which have probably absorbed genes from corn. These experiments clearly demonstrate that the character of the autoploid derivatives depends primarily upon the genic composition of the progenitor.

The duplication of the chromosomes of *Z. mexicana* appears to have taken place only once in the wild and, as in Randolph's chimera, to have resulted in the change from annual to perennial habit. This recalls the situation in the series of *Eragrostis* species, in which the perennial character is correlated with the degree of autoploidy attained.

In the closely related genus *Tripsacum*, another intraspecific difference in chromosome number has been reported by Mangelsdorf and Reeves (1939). Forms of *T. dactyloides* (L.) L. from three localities in Kansas and Texas had 18 pairs of chromosomes, and six other races ranging from Connecticut to Texas had 36 pairs. These were considered to be autoploids of comparatively recent origin, but on account of the complex relationships of *Tripsacum* species, this hypothesis cannot be considered confirmed as yet.

6. *Dactylis Aschersoniana* Graebn., $n = 7$, and *D. glomerata* L., $n = 14$. The grass genus *Dactylis* has usually been thought to contain only one species, *D. glomerata* L. Various forms of it have been described, however, in one category or another. One of these is the shade form, first recognized as a variety in the 1830's. Graebner, in 1899, elevated this form to a species, *D. Aschersoniana*, after verifying its systematic distinctness and constancy by garden experiments. This species, however, has not been generally accepted by taxonomists, because it differs from *glomerata* principally in characters which would be suspected of being environmental modifications induced by the dense shade of the beechwood type of forests in which it grows.

The characters that mark *Aschersoniana* are its rhizomes, slender stems, lax habit, thin, bright-green leaves, smooth herbage and glumes, and 5 to 6 florets in the spikelet, in contrast with *glomerata*, which is densely tufted, strict, and coarse, with bluish-green scabrous herbage, and 4 or 5 florets per spikelet. Turesson (1922*b*) cultivated the shade form from the Danish and southern Swedish beech woods and found that it approached *glomerata* in appearance when it was grown in open sun. The two remained distinct, however, and although the differences were not large, he concluded that the shade form was not a modification, but a shade ecotype of *glomerata*.

Cytological investigations further clarified the picture in this agriculturally important group. Levan (1930), working in part with Turesson's material, and Kattermann (1930) independently found that *Aschersoniana* is diploid, with $n = 7$, and *glomerata* is tetraploid, with $n = 14$ chromosomes. Müntzing (1933) verified the difference in chromosome number on a sample of 33 plants from other localities. Triploid natural hybrids occur where the two meet (Levan, 1930; Müntzing, 1937). At two localities near Svalöf, Müntzing found a total of 12 hybrids growing with their parents. These were morphologically intermediate between *Aschersoniana* and *glomerata*, and were pollen sterile, but they could be backcrossed to both parents.

Biosystematically, therefore, *Aschersoniana* and *glomerata* are good ecospecies of one cenospecies. They differ sufficiently from each other to be distinguishable, and they occupy ecologically distinct habitats in very different, but overlapping, geographical ranges. The diploid *Aschersoniana* is of limited distribution, for it grows in beech forests from southern Scandinavia to Germany and Poland. The tetraploid *glomerata* has a much more extensive range throughout Europe into northern Asia and northern Africa. It has also been introduced almost around the world. The exact natural distributions of the two remain to be worked out through chromosome counts.

Dactylis glomerata is generally accepted as an outstanding example of an autoploid that has arisen from a present-day species. Before it can be accepted as an unqualified autoploid derivative of *Aschersoniana*, however, other forms from relatively undisturbed natural habitats in Europe must be investigated, that may have played a role in the evolution of *glomerata*. For example, *D. abbreviata* Bernh. (*D. glomerata* var. *abbreviata* Drejer), which grows on open, sunny hillsides or sandy shores, is conceivably a progenitor. If this is another diploid, a hybrid between it and the shade-loving *Aschersoniana* should be able to produce, through amphiploidy, a species with the morphologic and ecologic characteristics of *glomerata*. Still other possibilities may reasonably be suggested, such as the Mediterranean *D. hispanica* Roth. The whole matter of the origin of *glomerata* awaits a thorough cytogeographic survey of the complex.

Whether or not *Dactylis glomerata* is autoploid or amphiploid, the relation between the degree of polyploidy and the environment is important. As in previous examples, a change in chromosome number is correlated with the occupation of a different environment. The diploid in *Vaccinium* occurs in the drier situations, that in *Eragrostis* in the moister, but here in *Dactylis*, so far as is known, it is confined to the shade.

7. *Biscutella coronopifolia* L., $n = 9$, and *B. laevigata* L., $n = 18$ (Manton, 1934, 1937). These members of the Cruciferae have been quite thoroughly investigated, but the full biosystematic importance of their story has not been generally understood as yet. Manton linked her excellent cytogenetic results with the most recent taxonomic monograph of the genus by Machatschki-Laurich, which, unfortunately, radically upheld a number of Alexis Jordan's elementary species. Manton, herself, found the experimental results in considerable disagreement with the taxonomic conclusions of Machatschki-Laurich. In the following discussion, therefore, Manton's results are compared directly with the less radical treatments of the genus found in the widely used regional floras of central Europe, in order to help clarify the biosystematic problems.

The diploid forms ($n = 9$) have a limited and discontinuous distribution like that of a relict species, being practically confined to unglaciated areas along the principal river systems of central Europe. Here they occur at low elevations in hot, dry places in an arc from southern France, across central Germany, to the middle Danube. Manton's extensive crossing experiments show that they produce F_1 hybrids freely, and the chromosomes of all are homologous. Accordingly, the biosystematic conclusion that these are probably members of one ecospecies coincides with the taxonomic one based on morphology and found in most regional floras. The earliest available name for this diploid is *B. coronopifolia* L., which is usually treated as a subdivision of *B. laevigata*. Previously, this name has been attached only to the diploid forms of France and the Mediterranean.

In the mountains near Vienna grows a diminutive diploid ecotype of *B. coronopifolia* called *B. laevigata* var. *mollis* Schur. (*B. minor* Jord.). It occurs with a definitely alpine flora above tree line in the Austrian Alps outside the area covered by the Alpine ice sheet of the last glacial period. This indicates that it differs physiologically from the diploids of the lowlands, which definitely are found on the hottest sites available in their districts. Manton reports that when it is brought to a lowland garden, it flowers very sparingly and is difficult to keep alive. This is characteristic behavior for many alpine ecotypes. It was interfertile with the only lowland race of *coronopifolia* with which it was tried, thus proving, in spite of its morphological differences, to be only an ecotype, and not a distinct species as suggested by Manton (1937).

A subalpine ecotype might be expected to interconnect the alpine and lowland ecotypes of *coronopifolia*, unless it has been exterminated. Manton's papers leave this in doubt, because purely morphological, rather than ecological, units were used as a basis for ecological discussions, but possibly *B. laevigata* ssp. *austriaca*, which is morphologically similar to the alpine ecotype and has a similar distribution but at lower elevations, may represent such an ecotype.

Aside from the above-mentioned alpine ecotype of *coronopifolia*, which is limited to the Austrian Alps, all other forms of *Biscutella* from high elevations in central Europe are tetraploid ($n = 18$). In contrast with the disjunct distribution of the diploids, the area occupied by the tetraploids is both extensive and continuous, spreading, with few exceptions, from the western Alps and the mountains of Italy to the northern Balkans. The tetraploids form a distinct ecospecies, for they are interfertile among themselves and only partially fertile with the diploids. This species is *B. laevigata* L., the type of which came from well within the area of the tetraploids and is morphologically identifiable with them (Manton, 1937).

Biscutella laevigata is cytologically unbalanced, for 19.4 per cent of a sample of 67 plants had $2n = 34$, 35, and 37, instead of the regular number of $2n = 36$ chromosomes. In meiosis many chromosomes associate in groups of four, and the presence of trivalents and univalents explains the oscillations in chromosome number. In spite of this chromosomal unbalance, the tetraploid species is a good colonizer. This faculty is correlated with its ability to proliferate root buds in cultivation, a character not shared with the diploids, and a principal difference between the two species. This improvement in the perennial quality of the tetraploid over the diploid recalls the development of the perennial habit in tetraploid teosinte.

Manton's data demonstrate that *coronopifolia* and *laevigata* are distinct ecospecies in spite of the fact that there are only slight morphological differences between them. Furthermore, they are the only members of their cenospecies. The other species of *Biscutella* have 6 or 8 pairs of chromosomes and are all Mediterranean.

From all the evidence it appears that tetraploid *Biscutella laevigata* is an autoploid of the diploid *coronopifolia*. The two are well fitted to different environments. The presence of both alpine and lowland ecotypes in the diploid species makes it possible that the tetraploid could have arisen through chromosome doubling in a hybrid between them, but it would still be an autoploid according to our definition.

8. *Tradescantia canaliculata* Raf., $n = 6$ and 12, and *T. occidentalis* (Britt.) Smyth, $n = 6$ and 12 (Anderson and Woodson, 1935; Anderson and Sax, 1936). The Tradescantias of the United States have been widely cited as instances of autoploidy. Because of their complex morphological

and cytogenetic relationships, the evidence is not so clear as in the previous instances.

The spiderwort of eastern meadows, *T. canaliculata,* and the one of western prairies, *T. occidentalis,* each have diploid and tetraploid forms. Out of 30 samples taken of *canaliculata* from Texas to Michigan, 5 were diploid as against 25 tetraploid, a fact which shows a preponderance of tetraploids in this species. These chromosomal races cannot be distinguished by any other character and are cohabitant.

The situation in *occidentalis* is more obscure. It seems to be predominantly tetraploid, because 9 out of 11 samples taken over the area from North Dakota to Texas and Arizona were tetraploid. The two diploids were from Texas and Oklahoma. But another diploid species of the prairies, *T. bracteata* Small, is morphologically similar to *occidentalis,* and it appears possible that it is the diploid counterpart of tetraploid *occidentalis,* and that the two diploids referred to the latter may be outlying forms of it.

The morphological differences between the species of *Tradescantia* are not very great. The sepals of *canaliculata* are glabrous except for a terminal tuft of glandless hairs, whereas those of *occidentalis* are glandular-pubescent and lack the tuft. Other minor characters also distinguish these species, but they interbreed on both the diploid and tetraploid levels, as is shown by the production of both natural and artificial hybrids.

The pollen fertility of the diploid species of *Tradescantia* was found usually to exceed 90 per cent, but in the interspecific hybrids it was reduced to from 40 to 74 per cent. These hybrid fertilities appear to be very high as compared with those of interspecific hybrids in general, and are higher than any which the present authors have observed in hybrids between very closely related ecospecies of one cenospecies.

The hybrids between *canaliculata* and *occidentalis* were partially fertile on each chromosome level. Since the tetraploid forms of these species are generally considered to be autoploid, their interspecific hybrid combines two genomes of each species and actually is an amphiploid of the diploid forms. The homology of the parental chromosomes prevents the amphiploid from being morphologically constant, and continual segregation occurs. This case is rather comparable with that of *Aquilegia Janczewskii* (see table 12, p. 72), except that the parents are still more closely related. Although the bulk of these two species doubtless consists of autoploid forms, many poorly defined mixtures of amphi- and autoploids must exist in the zone where their tetraploids occur together. The "introgressive hybridization" which Anderson and Hubricht (1938) described between the tetraploids of these two species refers to such mixtures.

A widespread condition of autoploidy, combined with poor genetic barriers between ecospecies of one cenospecies, may produce a very involved taxonomic situation, namely, that of the polyploid complex. Such a condition existing in *Tradescantia* caused Anderson and Woodson to recognize eighteen species in the United States. These can be intercrossed and are referable to one cenospecies, *T. virginiana* L.[1] A biosystematic analysis of the morphologic, geographic-ecologic, and cytogenetic data indicates that the members of this cenospecies occupy five sorts of edaphic niches, in each of which diploid and tetraploid forms have evolved. A somewhat generalized classification of these niches and their component units is as follows:

A. Moist woods and thickets
  Diploid: *Ernestiana* A. et W.; *edwardsiana* Tharp
  Tetraploid: *virginiana* L.; *subaspera* Ker-Gawl; *ozarkana* A. et W.
B. Meadows and bottoms
  Diploid: *canaliculata* Raf.; *paludosa* A. et W.
  Tetraploid: *canaliculata* Raf.
C. Drier wooded hillsides
  Diploid: *hirsuticaulis* Small
  Tetraploid: *Tharpii* A. et W.; *longipes* A. et W.
D. Sandy prairies
  Diploid: *bracteata* Small; *occidentalis* (Britt.) Smyth
  Tetraploid: *occidentalis* (Britt.) Smyth
E. Sandy areas in Gulf region
  Diploid: *subacaulis* Bush; *humilis* Rose
  Tetraploid: *hirsutiflora* Bush; *roseolens* Small

The units in each of these ecologic groups except the last are considered closely related by Anderson and Woodson, although this is not always obvious from their key or the order in which the species are treated. This situation is unique, with a complete set of diploids and tetraploids present in each major habitat occupied by the genus.

Unless chromosome differences offer strong barriers to interbreeding, it is uncommon for more than one ecospecies of a cenospecies to occupy a particular environment. In *Tradescantia,* then, if all the above-listed units belong to one cenospecies, as the incomplete crossing data seem to indicate, one should expect to find only one diploid and one tetraploid ecospecies in each of the five environments. The genetic and ecologic data, however, are insufficient to enable one to evaluate fully the biosystematic pattern in this complex.

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[1] A cenospecies should be known by the name of the earliest recognized species it contains. Since a cenospecies will be known by the name of an ecospecies within it, one must indicate what kind of species is meant. For example, the ecospecies *T. virginiana* L. is the type species of this complex, and we assign a number of taxonomic species to the cenospecies *T. virginiana* L.

Some of the larger units, for example *virginiana*, *subaspera*, *canaliculata*, and *occidentalis*, cover an area in which two or three regional ecotypes might be expected to exist; but only one, *Tradescantia subaspera* var. *montana* (Shuttlew.) A. et W., of the Appalachians, is morphologically distinct enough to have received recognition. The rather extensive studies already made are clearly indicative that when the entire cenospecies *T. virginiana* has been fully investigated, most interesting correlations between plant and habitat will be disclosed.

9. *Cuthbertia graminea* Small, $n = 6$, 12, and 18 (Giles, 1942). This case was interpreted as one of autoploidy, but the arithmetical progression of the chromosome numbers suggests that amphiploidy is the more probable pattern.

The genus *Cuthbertia* is a close relative of *Tradescantia* and contains, according to Small, the three species *C. graminea*, *C. rosea* (Vent.) Small, and *C. ornata* Small. All three are endemics of the southeastern United States, occurring on the coastal plain from North Carolina to Florida, and differ in their ecologic requirements. Only *graminea* is found throughout the entire area, *ornata* being limited to Florida, and *rosea* extending the farthest north and west, with a range extensively overlapping that of *graminea*. *Cuthbertia rosea* is diploid, but the number of chromosomes in *ornata* is unknown.

The three species are very closely related; Anderson and Woodson (1935) treated them as only varietally distinct members of *Tradescantia rosea* Vent. These authors emphasized, however, that they were distinct from all members of the cenospecies *T. virginiana*, which compose the bulk of the genus. Giles was unsuccessful in various attempts to cross members of the two groups. This indicates that *Cuthbertia* and *Tradescantia* belong to different comparia. This barrier to interbreeding seems to be a more important generic criterion than the difference in chromosome morphology stressed by Giles.

In an extensive cytological survey of *graminea*, plants from twelve localities were found to be diploid, and from forty-six localities, tetraploid. Two random hexaploids and a single triploid were also discovered. The diploid element occurs in a limited area in North Carolina along the Fall Line, in which the tetraploid is not found. The latter element occupies an area many times larger throughout the South Atlantic coastal plain on soils of more recent origin.

Morphologically, the tetraploids are larger and somewhat more variable than the diploids. Measurements show that diploid and tetraploid *graminea* have statistically significant differences in such a character as leaf width, which is used to distinguish *rosea* and *graminea*. In addition, the tetraploids are taller and have twice as heavy seeds.

Giles submits as evidence of autoploidy the morphological similarity between diploid and tetraploid *graminea*, the presence of quadrivalents in the tetraploid, and the similarity in chromosome morphology. None of these can be considered proof of autoploidy, as was mentioned earlier. The chromosome morphology of even the closest relatives is not known. The facts cited by Giles consequently show only that autoploidy is possible, but not that it is probable.

The biosystematic status of the species of *Cuthbertia* is still unknown. Four units, *C. rosea*, diploid and tetraploid *graminea*, and *ornata*, are morphologically and ecologically recognizable. At present, however, we do not know whether the diploids are interfertile or intersterile, and much depends upon this information.

Consequently, this is a case in which the earliest-known facts pointed to autoploidy, but where an assembling of additional data about the closest relatives is likely to revise the first impression. On morphological grounds, tetraploid *graminea* could have arisen as an amphiploid from diploid *graminea* and *rosea*, and even other alternatives are possible. Until the chromosome number of *ornata* is known, it is too early to express an opinion on the origin of the tetraploid form of *graminea*.

10. *Galium Mollugo* L., $n = 11$ and 22, and *G. verum* L., $n = 11$ and 22 (Fagerlind, 1937). The bedstraws of Europe constitute a polyploid complex with autoploidy and amphiploidy interwoven into a pattern very similar to that observed in American *Tradescantia*, except that the bedstraw species are genetically much better separated. From Fagerlind's very comprehensive investigations, however, a more complete biosystematic background is available for European *Galium* than for *Tradescantia*.

Within 13 of the 48 species of *Galium* investigated, more than one chromosome number was found. Extensive crossing experiments were performed between *Galium Mollugo*, *verum*, and *lucidum* L., each with $n = 11$ and 22, *G. Kitaibelianum* Schult. and *G. silvaticum* L., each with $n = 11$, and *Asperula glauca* (L.) Bess. (*Galium glaucum* L.), with $n = 11$ and 22. Only one interspecific hybrid combination was obtained on the diploid level, and this gave two F_1 hybrids between *Asperula glauca* and *Galium verum*. The extensiveness of these experiments is shown by the crossings between diploid *Mollugo* and diploid *verum;* 848 flowers were emasculated and pollinated to afford 1696 possible seed, but only 124 of these started to develop, only 41 ripened, and none would germinate. The crossing attempts therefore showed that the diploid *Galium* species employed are genetically exceedingly distinct.

In this group, it is almost impossible to obtain hybrids not only between diploids of different species, but also between diploids and tetraploids even when they are referred to the same species. The very few hybrids obtained in the latter cases were on the diploid as the female parent and were not triploid as expected, but tetraploid. This indicates that diploid ovules are occasionally produced in diploid races, and reveals a mechanism whereby polyploidy can take place. In genetic and ecologic respects the chromosomal races are specifically distinct, but since they lack morphological differences, it is impracticable to recognize them taxonomically.

These conclusions are well illustrated in the two most common and best-investigated species, *Mollugo* and *verum.* These are cohabitant almost throughout their range, except that *Mollugo* has pushed a little farther north and east. Both are abundant in meadows and woods and along roadsides almost throughout Europe and southwestern Asia up to about 2000 meters elevation. They differ by half a dozen distinctive characters, such as the open inflorescences and white flowers of *Mollugo* in contrast with the congested inflorescences and deep-yellow flowers of *verum.* The strong genetic barriers between them permit them to grow side by side without intermixing.

The diploid races have been found only in the southeastern part of the distribution of both species, that is, in the Balkan peninsula and the Carpathians, where they occur together with a few tetraploids. Diploid *verum* is also Transcaucasian. Toward the north and the Atlantic only tetraploids occur (see maps in Fagerlind, 1937, p. 354).

In areas where both diploid and tetraploid *Mollugo* grow, they are entirely cohabitant, but the diploid form of *verum* appears to inhabit meadows, whereas the tetraploid is found in the drier localities. The tetraploids of both species, as would be expected from their distribution, were found to be more resistant to cold than the diploids.

Hybrids have been obtained with difficulty between the tetraploid races. Among 1494 possibilities for fertilization after hand pollination, Fagerlind observed 146 seeds developing, but only 22 ripened. These produced 12 F_1 plants, of which only 5 were able to develop.

Natural hybrids between the tetraploid races of *Mollugo* and *verum* are common throughout central Europe, but rare in the Scandinavian peninsula. These may be regarded as delayed amphiploids of the diploid members of these species, both of which occur distant from the localities in which the hybrid is usually produced. This amphiploid, which is intermediate in flower color and other characters, has received taxonomic recognition as *G. ochroleucum* Wolff. The chromosomes of the parental species are at least partly homologous, for quadrivalents occur with the same frequency in the amphiploid as in its autotetraploid parents. For this reason, *Galium ochroleucum* is comparable with *Primula kewensis* (see table 12, p. 72). Fagerlind showed that its offspring segregate, and that the species is only partially fertile, a situation that might be expected from the homology of its chromosomes and the incompatibility of its diploid ancestors.

The amphiploid *G. ochroleucum* is somewhat less fertile with its tetraploid parents than with itself, but it has become a bridge for gene flow from one parental species to the other. The segregation of its offspring and its backcrosses to the tetraploid parents in the wild have caused much taxonomic confusion, for some of the segregates have been referred to one species, and others to another.

The major part of the tetraploid forms of *Galium* are probably true autoploids, since the species are unable to hybridize on the diploid level. The tetraploids are also the exact morphological counterparts of their diploid relatives and occur with them. As was suggested in the discussion of *G. ochroleucum*, one or two quadrivalents are fairly common in their chromosome sets, the remainder of the chromosomes pairing as bivalents.

It is indeed remarkable that with these strong genetic barriers between them, several of the morphologically differentiated species do not occupy the same environment in the way that *Mollugo* and *verum* do. For the most part they follow the distribution pattern of ecospecies, having different geographic ranges, or occurring in different edaphic habitats.

The homology of the chromosomes in the species of *Galium* suggests that phylogenetically these are closely related. The strong barriers to interbreeding are therefore probably of a secondary nature, possibly indicating only an unbalance in the embryo-endosperm relation in the developing seed.

The biosystematic picture of the European Galiums is therefore one of well separated cenospecies. They have produced a series of autoploids that are partially cohabitant with the diploids. Genetically, the autoploids are well separated from one another and from their diploid progenitors. However, the cohabitant tetraploid races of two species, *Mollugo* and *verum*, have been able to produce a morphologically intermediate but partly inconstant amphiploid, *ochroleucum*, which acts as a means for gene interchange between them. This situation has built up a critical tetraploid complex which stands out from the otherwise very distinct speciation in the genus.

CONCLUSIONS

As one surveys these examples of natural autoploids, several important contrasts between them and natural amphiploids stand out. First, they differ less from their progenitors in their ecologic requirements. In some instances the autoploid is even completely intermixed with its parent, and commonly it is found in closely adjacent territory and overlapping with the parent in distribution.

Sometimes autoploids are morphologically indistinguishable from their progenitors, whereas amphiploids are usually clearly recognizable. When autoploids occupy distinct habitats, however, they are prone to be morphologically distinct, too; but these differences are usually of lesser magnitude than in the case of amphiploids.

In the evolution of some groups of plants, amphiploidy has been superimposed upon autoploidy. This has happened in *Tradescantia*, *Zauschneria*, and probably the American species of *Vaccinium*. In *Galium*, where some mechanism prevents the species from intercrossing on the diploid level, amphiploidy could not take place except through the autoploids. In such groups, the combinations of autoploidy and amphiploidy produce an intricate complex of forms. In a group like *Galium*, also, the complexity is increased because the diploid progenitors of the amphiploids have some homologous chromosomes, permitting a certain amount of segregation in the amphiploids. In the American forms of *Crepis* (Babcock and Stebbins, 1938), such a pattern is made even more complex by apomixis, which preserves a greater number of hybrid forms than is otherwise possible.

A critical examination of the ecologic-taxonomic status of a polyploid complex and its relatives is necessary before one can determine whether evolution in the group has followed autoploidy or amphiploidy. For instance, with all the data now on hand concerning *Dactylis* and *Cuthbertia*, their status as autoploids is still open to question, for the role in their evolution of related rare endemics from extreme habitats has not been settled. In many complexes composed of several closely related species, as *Tradescantia*, *Cuthbertia*, *Zauschneria*, and *Galium*, crossing experiments are required before the type of ploidy involved can be determined.

An extreme view in regard to the criteria which must be met before autoploidy is accepted is held by Böcher (1936), who questioned the autoploid nature of even such tetraploids as *Empetrum hermaphroditum*, *Vaccinium uliginosum*, and *Campanula rotundifolia*. He regarded these as morphologically too distinct from their diploid relatives to be reasonably accounted for as autoploids, a position defended by the argument that experimentally produced *Solanum* autoploids show no such differences. This view is not valid, however, since from the characteristics of natural polyploids it is known that different groups of plants do not react in the same way to chromosome doubling. No one has thus far produced autotetraploid *Empetrum hermaphroditum*, *Vaccinium uliginosum*, or *Campanula rotundifolia* experimentally, but even though this were done, one could hardly expect them to be identical with the natural forms, because of probable genic differences between the parents in the two sets of cases. Böcher's suggestion that additional diploid ancestors of these tetraploids may have existed in the past, though plausible, is not open to experimental test, and its acceptance would almost exclude any but experimentally produced individuals from the ranks of admissible autoploids.

Simple doubling of the chromosomes, with all their included genes, clearly is a process that may alter the physiologic balance of a plant, so that it can occupy an ecologic niche to which its progenitor was not adapted. From then on, differential selection should eventually make diploid and tetraploid forms morphologically distinct also, so that they would become distinct species. The remarkable alteration from annual to perennial habit in teosinte and *Eragrostis* is indicative of the changes that can be produced by such a doubling.

IX

TAXONOMIC AND EVOLUTIONARY ASPECTS OF AMPHIPLOIDY AND AUTOPLOIDY

As objects of taxonomic classification, species of polyploid origin present some of the most perplexing problems. In groups in which either amphiploidy or autoploidy alone takes place, the natural units may sometimes be disclosed by careful analysis of morphological characters, as, for example, in the *Iris versicolor* complex (Anderson, 1928). When, however, one kind of ploidy is superimposed upon another, classification becomes so difficult that cytogenetic results must be correlated with morphologic and geographic data if the interpretations of the taxonomist are to have permanent value.

The application of biosystematic principles in taxonomy may be expected to advance greatly our understanding of all complex groups. The more adequately classification can express the relations between organisms, the relations between them and their environments, and the dynamics of their evolution, the more nearly will taxonomy become the coordinating branch of the biological sciences. The importance of amphiploids and autoploids to taxonomy and their role in the evolutionary processes may now appropriately be considered.

Amphiploids. The amphiploid is effectively set off from its parents by internal barriers, for both the difference in chromosome number and the new genetic balance usually prevent free interchange of genes between them. It is therefore a genetic species from its inception, and the taxonomist may anticipate from the nature of its origin that morphological characters may be found to distinguish it as a taxonomic species as well.

The amphiploid combines the morphological features of its parents and will therefore generally appear to be intermediate. The presence of such an intermediate species tends to obscure the differences between even the parental species, which may have been clearly distinct before the amphiploid arose. Consequently, we find that in such groups taxonomists who rely wholly upon morphology hesitate to recognize the amphiploids, or sometimes even the parents, as species. This is shown in table 13, which lists natural amphiploids discussed in previous chapters, together with the species with which they have been confused.

Amphiploidy opens a new avenue of evolution in any group mature enough for this type of development. In the *Brassica-Sinapis-Raphanus* comparium, for example, evolution on the diploid level results in a considerable series of well separated species, including *Raphanus sativus*, $n = 9$, *Brassica nigra*, $n = 8$, *B. oleracea*, $n = 9$, and *B. campestris*, $n = 10$. By the addition of these basic genomes, an amphiploid level has been built upon them, represented by *Brassica carinata*, $n = 17$, *B. juncea*, $n = 18$, *B. Napus*, $n = 19$, and *Raphanobrassica*, $n = 18$. The beginning of a hexaploid level is found in two artificial amphiploids, with $n = 27$ and 29.

TABLE 13

AMPHIPLOIDS THAT HAVE BEEN CONFUSED WITH ONE OR BOTH PARENTS

Amphiploid	Confused with
Spartina Townsendii	S. alterniflora and stricta
Phleum pratense	P. nodosum
Poa annua	P. exilis and supina
Iris versicolor	I. virginica
Eriogonum fasciculatum ssp. foliolosum	E. fasciculatum sspp. typicum and polifolium
Rumex Acetosella	R. angiocarpus and tenuifolius
Brassica Napus	B. oleracea and campestris
Galeopsis Tetrahit	G. speciosa and pubescens
Penstemon neotericus	P. laetus and azureus
Madia citrigracilis	M. gracilis
Artemisia Douglasiana	A. Suksdorfii and ludoviciana

The rise of a successful amphiploid often changes the biosystematic status of its parents. Even though the parents previously were unable to exchange genes and belonged to two cenospecies, the advent of the amphiploid merges them into one.

AUTOPLOIDS. Autoploids are taxonomically even less distinct than amphiploids. The latter can be recognized by the traditional taxonomic characters, once the correlation between such characters and the cytogenetic history has been pointed out. In autoploids, however, the kind of technical differences taxonomists hope to find are usually missing. When autoploids are detected by taxonomists, they sometimes are classified as *formae*, because they have quantitative differences, as in size of leaves and other organs. In table 14 the presence or absence of morphological and ecological distinctness of the autoploids discussed in chapter VIII is summarized in order to give a general picture of the nature of the differences between autoploids and their progenitors.

There is no doubt that most of these autoploid units play the same roles in nature as do well recognized species, for most of them have distinct ecologic preferences and do not interbreed freely with their diploid counterparts. They are therefore important units for the ecologist and the evolutionist. Wherever morphological differences are available, the autoploids should be recognized as taxonomic species, even though the differences are less distinct than those between species on one chromosome level.

TABLE 14

DISTINCTIONS BETWEEN AUTOPLOIDS AND THEIR PROGENITORS

Autoploid	Morphologic differences	Ecologic preferences
Zea perennis	Perennial rhizomes	Unknown
Dactylis glomerata	Several minor characters	More sunny places
Eragrostis	Larger size, longer duration of life	Drier habitats
Tradescantia	None	None
Biscutella laevigata	Stolons developed	Higher altitudes
Galax aphylla	Essentially none	None
Vaccinium uliginosum	Larger leaves, taller	Less arctic
Empetrum hermaphroditum	Bisexual, more fruits	More arctic
Galium Mollugo and G. verum	None	Somewhat more northern

EVOLUTIONARY SEQUENCE

In the evolution of plant groups different stages can be recognized. A rather early stage is probably found in *Aquilegia*, a genus containing many very distinct ecotypes, but few genetically well separated ecospecies. Another example is *Potentilla glandulosa* Lindl. and its allies, of the section *Drymocallis* (Clausen, Keck, and Hiesey, 1940). Groups of this sort are very flexible in adapting themselves to changing conditions, for they contain few barriers to the interchange of genes. Such groups are often able to inhabit areas with widely contrasting climates.

The opposite extreme is reached when inflexible genetic barriers are interposed between species, as in monotypic cenospecies of one comparium. A marked depauperization of its variation has doubtless taken place before a comparium reaches this stage. The section *Anisocarpus* of *Madia*, to which *nutans* and *Rammii* belong, is an example of a group that has come to such maturity. At this stage the comparium is ripe for amphiploidy, and the genome, rather than the individual gene, becomes the building block for evolution.

The institution of amphiploidy rejuvenates a comparium even after its species have become isolated and relatively homogeneous, because the amphiploids serve as intermediaries for gene exchange between its members. Several distinct cenospecies of one comparium may thereby be merged into one, and the group may then experience revitalization.

A group like *Aquilegia* may be youthful in genetic constitution but old in terms of time. The genus *Galium*, on the other hand, behaves as though it were genetically senescent, but actually it may be younger than *Aquilegia*. The existence of a great variety of races, and delay in the institution of genetic barriers between them, keep a group youthful and flexible. From the biosystematic view, youthfulness and age are therefore relative terms.

The biosystematic units—ecotypes, ecospecies, cenospecies, and comparia—represent stages in an evolutionary sequence. A group of plants may successively pass through them all. For example, following ecologic isolation, ecotypes may develop, and then one or several of these may be separated from the others through genetic barriers, giving rise to ecospecies. The complex genetic basis of these barriers suggests that they may develop very gradually, for time is an important factor in such differentiation. Then, following the disappearance of closely related forms, and an increase in the distinctness of the genetic barriers, different cenospecies are set off. Finally, the advent of complete genetic isolation among the cenospecies elevates them to the status of separate comparia. From the level of the ecospecies to that of the comparium, the many avenues for obtaining new supplies of genes through hybridization are closed, one by one, and evolutionary progress becomes increasingly more dependent upon the internal potentialities of the group.

The steps may be partly retraced with the institution of amphiploidy, so that cenospecies, for example, become reunited. Each development of this sort offers fresh evolutionary opportunities. But eventually the comparium exhausts its possibilities along this path, and then partial or complete vegetative propagation by apomixis may be instituted. If this occurs, the variation may suddenly increase appreciably through the preservation of various kinds of otherwise sterile hybrids. The North American polyploid and apomictic species of *Crepis* exemplify this stage of development (Babcock and Stebbins, 1938). In such comparia, the extensive morphological recombinations often give the impression that all the species have merged into one.

In comparia composed solely or mostly of ecotypes, a free exchange of genes is possible, and the individual gene is the evolutionary building unit. As a decimation of biotypes takes place, producing gaps in the series of forms, ecospecies and cenospecies emerge, and the genome gradually evolves. This may all take place on the diploid level. Dysploidy in low chromosome numbers probably represents but a variation of this pattern.

After distinct cenospecies have evolved, the genome has replaced the gene as the important building block, and the comparium is ready for amphiploidy. From this point on, the retention of essentially unbroken parental genomes is necessary for success, and almost all evolution is by the addition of genomes in various combinations. Species now arise abruptly, pyramiding their chromosome numbers to form successively higher levels. By adding the genomes of old species, the amphiploids also combine the ecological requirements of the parents, and may therefore be adapted to colonize new environments. Autoploidy is a variation of this pattern, resulting in less diverse products. Some of these aspects were discussed by Stebbins (1940), who pointed out that the amphiploids are the really expansive species.

In the course of time, selection may again deplete the variation among the polyploids, and the group may become senile. At this point, if apomixis evolves, it may preserve various kinds of F_1 hybrids irrespective of sexual fertility or chromosomal pairing. This is probably the last evolutionary upsurge of the group before its gradual extinction, but most comparia perhaps never reach this stage.

In such ways as these, living things undergo a pulsating differentiation. Their fortunes ebb and flow. The processes of gene mutation, recombination, and selection build ecotypes adapted to different climates. Then barriers to interbreeding separate ecotypes into ecospecies. A great decrease in genetic flexibility of these units marks the evolution of distinct cenospecies. Amphiploidy may then appear as a vehicle for furthering evolutionary progress at a time when stimulation is most useful.

The study of the circumstances that lead to the synthesis of amphiploids brings into focus key facts concerning plant relationship that clarify the origin of much of the otherwise perplexing biological variation. The role of the various biosystematic elements in the evolutionary development of a group becomes evident, and the rich complexity of so many genera is found to express but the logical operation of fairly simple laws.

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INDEX

A

Aase, H. C., 88
Aegilops, 67
  sharonensis, 90
  speltoides, 89, 90
  speltoides × *sharonensis*, 90
  umbellata, 90
  umbellata × *Haynaldia villosa*, 90
  uniaristata, 90
Agropyron, 67
alloploidy, definition of, vii
allopolyploidy, vii
amphidiploidy, vii
amphiploid-autoploid mixtures:
  in *Galium*, 144
  in *Rumex*, 124
  in *Tradescantia*, 141
  in *Vaccinium* of eastern United States, 134
  in *Zauschneria*, 126
amphiploids:
  affecting biosystematic status of their parents, 86, 94, 98, 146–147, 150, 151
  classification of, 71–72
  derived from interspecific hybrids, 70
  ecological adaptability of, 128, 153
  experimental:
    Aegilops speltoides × *sharonensis*, 90
      umbellata × *Haynaldia villosa*, 90
    Aquilegia Janczewskii, 77, 116
    Brassica chinensis × *carinata*, 94
      chinensis × *Raphanus sativus*, 93
      napocampestris, 94
      Napus, 92
    Crepis capillaris × *tectorum*, 80
      rubra × *foetida*, 79
    Digitalis mertonensis, 84
    Erophila duplex × *quadruplex*, 82
    Galeopsis Tetrahit, 81
    Gossypium Stocksii × *arboreum*, 96
      Thurberi × *arboreum*, 95
    Lactuca indicata, 101
    Layia pentaglossa, 48, 81
    Madia citrigracilis, 24, 101
      nutrammii, 8, 100
    Nicotiana digluta, 98
      rupa, 98–99
      Tabacum, 96–97
      Vaviloviana, 99

amphiploids—*continued*
  experimental—*continued*
    Phleum pratense, 84–85
    Primula kewensis, 76–77
    Prunus domestica, 103
    Raphanobrassica, 91
    Rubus loganobaccus, the loganberry, 112–114
      maximus, 110–112
      strigosus × *ulmifolius rusticanus* (?), the veitchberry, 112
      trivialis × *strigosus*, the nessberry, 108–109
    Solanum luteum × *nigrum*, 99–100
    Triticale, 86–87
    Triticum monococcum × *Aegilops uniaristata*, 90
      turgidum × *Aegilops speltoides*, 89
  intergradation with autoploids, 70
  method of origin of, 118
  natural:
    Artemisia Douglasiana, 127–128
    Brassica Napus, 92
    Eriogonum fasciculatum ssp. *foliolosum*, 123
    Galeopsis Tetrahit, 82, 126
    Galium ochroleucum, 146
    Gossypium barbadense and *hirsutum*, 96
    Iris versicolor, 122
    Madia citrigracilis, 23–24, 41, 42, 127
    Nicotiana Tabacum, 97
    Penstemon neotericus, 126
    Phleum pratense, 86, 121
    Poa annua, 121
    Prunus domestica, 103
    Rubus maximus, 110
    Rumex Acetosella, 123–124
    Spartina Townsendii, 120–121
    Tradescantia canaliculata × *occidentalis*, tetraploid, 141
    Zauschneria californica ssp. *typica*, 124–126
  perpetuation of genetic balance in, 71 *et seq.*
  pseudo, 99
  quasi, 60, 80
  requirements for classification of, 73–74

amphiploids—*continued*
  requirements for success in, 68–69, 71–73, 117–118
  segregating:
    Aquilegia Janczewskii, 78
    Crepis rubra × *foetida*, 79
    Galium ochroleucum, 146
    Layia pentaglossa, 60
    Nicotiana rupa, 99
      Vaviloviana, 99
    Primula kewensis, 77
    Tradescantia canaliculata × *occidentalis*, 141
  success of, in the wild, 128 *et seq.*
  taxonomic recognition of, 149 *et seq.*
  through predoubling in parents:
    Galium ochroleucum, 146
    Rubus loganobaccus, 112
    Tradescantia canaliculata × *occidentalis*, 141
    Zauschneria californica ssp. *typica*, 126
amphiploidy:
  definition of, vii, 70, 117
  in evolutionary sequence, 151
  through gametic doubling:
    Brassica Napus, 92
    Crepis capillaris × *tectorum*, 80
      rubra × *foetida*, 79
    Digitalis mertonensis, 84
    Erophila duplex × *quadruplex*, 83
    Galeopsis Tetrahit, 82
    Lactuca indicata, 102
    Layia pentaglossa, 55, 81
    Madia citrigracilis, 29 *et seq.*, 101
    Madia nutrammii, 20, 100
    Nicotiana Tabacum, 97
    Phleum pratense, 85
    Raphanobrassica, 91
    Rubus maximus, 111
    Triticale, 88
  through somatic doubling:
    Aegilops speltoides × *sharonensis* (colchicine), 90
      umbellata × *Haynaldia villosa* (colchicine), 90
    Aquilegia Janczewskii (?), 77
    Brassica Napus, 92
    Gossypium Thurberi × *arboreum* (colchicine), 95
    Nicotiana digluta, 98
      Tabacum (hetero-auxin), 97
    Primula kewensis, 76

amphiploidy—*continued*
  through somatic doubling—*continued*
    Triticum monococcum × *Aegilops uniaristata* (colchicine), 90
      turgidum × *Aegilops speltoides* (colchicine), 89
Anderson, E., 122, 149
  and L. Hubricht, 141
  and K. Sax, 140
  and R. E. Woodson, 140, 143
aneuploidy, definition of, vi
apomixis, in evolutionary sequence, 152
application of biosystematic principles. *See under* biosystematics
Aquilegia, 151, 152
  as a cenospecies, 79
  chrysantha, 77
  chrysantha × *flabellata*, 77
  flabellata, 77
  formosa, 78
  Janczewskii, 77, 116
  natural and experimental intra- and intersectional hybrids in, 78
  pubescens, 78
Arnason, T. J., 136
Artemisia:
  Douglasiana, 127, 128, 150
  ludoviciana, 127, 150
  Suksdorfii, 127, 150
Arwidsson, Th., 132
Asperula:
  glauca, 144
  glauca × *Galium verum*, 144
autoploids:
  contrasted with amphiploids, 147
  differentiation of, 148
  experimental:
    Fragaria bracteata × *vesca* var. *rosea*, 74 *et seq.*
    Zea perennis, 136
  natural:
    Biscutella laevigata, 139
    Campanula rotundifolia (?), 134
    Dactylis glomerata, 137
    Empetrum hermaphroditum, 132
    Eragrostis albida and *pallescens*, 134
    Galax aphylla, 131
    Galium Mollugo, 144
      verum, 144
    Rumex graminifolius, 124
    Tradescantia canaliculata, 140
      occidentalis, 140
    Vaccinium uliginosum, 133

autoploids—*continued*
  natural—*continued*
    Zauschneria californica:
      ssp. *angustifolia,* 125
      ssp. *latifolia,* 125
    Zea perennis, 135
  origin of, 70
  recognition of, 131, 147–148
  segregation in:
    Fragaria bracteata × *vesca* var. *rosea,* 75
  taxonomic recognition of, 150 *et seq.*
autoploidy:
  definition of, vi, 70, 116
  multivalent association as an indicator of, 130
autopolyploidy, vi

B

Babcock, E. B., 80
  and M. Navashin, 79
  and G. L. Stebbins, 44, 81, 147, 152
  G. L. Stebbins, and J. A. Jenkins, 102
Bailey, L. H., 107, 113, 115
Baldwin, J. T., 131
barriers, genetic, in the evolutionary sequence, 152
Beasley, J. O., 95, 96
biosystematic units, 63 *et seq.,* 152
  applied in the classification of auto- and amphiploids, 71
  evolutionary sequence of, 152
biosystematics:
  definition of, iii
  principles of, 62
    application of, 66 *et seq.*
Biscutella:
  coronopifolia, 139
    ecotypes in, 139
  laevigata, 139, 151
    ssp. *austriaca,* 140
    var. *mollis,* 139
  minor, 139
Bleier, H., 87
Böcher, T. W., 134, 148
boysenberry, 115
Boza Barducci, T., and R. M. Madoo, 95
Brainerd, E., 105
  and A. K. Peitersen, 106
Brassica:
  alba, 94
  alboglabra, 93
  campestris, 92, 93, 117, 150
  campestris × *oleracea,* 92
Brassica—*continued*
  carinata, 93, 117
  chinensis, 93
  chinensis × *carinata,* 94
  chinensis × *Raphanus sativus,* 93
  comparium, 93, 94
  japonica, 93
  juncea, 93, 117
  napocampestris, 94
  Napus, 92, 117, 119, 150
  Napus × *campestris,* 94
  nigra, 93, 117
  oleracea, 92, 93, 94, 117, 150
  pekinensis, 93
  Rapa, 93
  verucastrum, 94
Brown, S. W., 106, 107, 108, 112, 113
Buxton, B. H., and C. D. Darlington, 84
  and W. C. F. Newton, 84

C

Camp, W. H., 134
  and C. L. Gilly, vi
Campanula rotundifolia, 134
cenospecies, definition of, vi, 64
  nomenclature of, 142
Ceratiola, 132
chimera, sectorial, diploid-tetraploid, in teosinte, 136
chromatin bridges:
  in *Layia pentachaeta* × *platyglossa,* 56, 57
Clausen, R. E., 96, 97, 98
  and T. H. Goodspeed, 98
Collins, G. N., and J. H. Kempton, 136
  and A. E. Longley, 136
comparium:
  definition of, vi, 64
  examples of:
    Aquilegia (one cenospecies), 79
    Brassica-Raphanus-Sinapis-Eruca, 92 *et seq.,* 94, 150
    Erophila (one cenospecies), 83
    Festuca-Lolium, 67
    Fragaria, 75
    Ginkgo, 65
    Lactuca (two comparia), 102
    Layia, 81
    Rubus, 115
    Triticum-Aegilops-Agropyron-Haynaldia-Secale-Elymus-Hordeum-Sitanion, 67
    Zea-Euchlaena-Tripsacum, 67
  maturation and rejuvenation of, 151

Corema, 132
Coville, F. V., 134
Crane, M. B., 112, 114
  and C. D. Darlington, 106, 112, 113, 114, 115
  and W. J. C. Lawrence, 103
Crepis:
  American species, 152
    origin of, 81
  capillaris, 80
  capillaris × *tectorum*, 80
  rubra × *foetida*, 79
  tectorum, 80
Cuthbertia:
  graminea, 143
  ornata, 143
  rosea, 143

D

Dactylis:
  abbreviata, 138
  Aschersoniana, 137
  Aschersoniana × *glomerata*, natural hybrids, 138
  glomerata, 137, 151
    var. *abbreviata*, 138
  hispanica, 138
Danser, D. H., vi, 63
Darlington, C. D., 71, 103, 116
Darrow, G. M., 105, 115
  and A. E. Longley, 115
Digitalis:
  ambigua, 84
  mertonensis, 84
  purpurea, 84
  purpurea × *ambigua*, 84
diploid gametes:
  in *Digitalis purpurea* × *ambigua*, 84
  in *Erophila duplex* × *quadruplex*, 83
  in *Layia*, 119
  in *Layia pentachaeta* × *platyglossa*, 55
  in *Madia*, 119
  in *Madia gracilis* × *citriodora*, 31
  in *Madia nutans* × *Rammii*, 15
  in *Rubus*, 106
Dobzhansky, Th., iii
Draba verna, 83
Duchesnea indica, 75
dyads:
  in *Digitalis purpurea* × *ambigua*, 84
  in *Erophila duplex* × *quadruplex*, 83
dyads—*continued*
  in *Lactuca indicata*, 102
  in *Layia pentachaeta* × *platyglossa*, 55
  in *Madia gracilis* × *citriodora*, 31
  in *Madia nutans* × *Rammii*, 16
  in *Raphanus sativus* × *Brassica oleracea*, 91
  in *Triticum vulgare* × *Secale cereale*, 88
dysploid pattern:
  in *Brassica* comparium, 93
  in *Erophila*, 83
  in the Madiinae, 1
dysploidy:
  definition of, vi
  through amphiploidy, 131
    in *Brassica* comparium, 94, 117
    in *Erophila*, 83
    in *Iris*, 122
    in *Madia*, 20
    in *Spartina*, 121

E

ecospecies, definition of, vi, 64
ecotype, definition of, vi, 63
Elymus, 67
Emerson, R. A., and G. W. Beadle, 136
Empetrum:
  hermaphroditum, 132, 151
  nigrum, 132
    forma *hermaphroditum*, 132
  rubrum, 132
Eragrostis, 151
  albida, 117, 134
  Cambessediana, 117, 134
  pallescens, 117, 134
Eriogonum fasciculatum:
  ssp. *foliolosum*, 123, 128, 150
  ssp. *polifolium*, 123, 150
  ssp. *typicum*, 123, 150
Erioxylum aridum, 96
Erophila:
  duplex, 83
  duplex × *quadruplex*, 82
  quadruplex, 83
  semiduplex, 83
  simplex, 83
  verna, 83, 131
Eruca sativa, 94
Euchlaena mexicana, 67
evolutionary levels, 65
evolutionary processes, 65–66

evolutionary sequence of biosystematic units, 151
experimental method in taxonomy, applied to:
  Aquilegia, 79
  Biscutella, 140
  Brassica comparium, 93, 94
  Dactylis glomerata complex, 138
  Erophila, 83
  establishing generic identity:
    of *Euchlaena mexicana,* 67, 136
    of *Hemizonia Wheeleri,* 67
    of *Madia nutans,* 4, 5, 67
  Festuca and *Lolium* placed in one comparium, 67
  Fragaria vesca complex, 74
  Galium Mollugo-verum complex, 146
  Gossypium comparium, 96
  Layia, 68
  Madia gracilis complex, 42–44
  Phleum pratense complex, 86
  Rubus:
    New England, 106
    Pacific coast, 107
  Tradescantia, 142
  Triticum comparium, 67, 88, 90
  Vaccinium uliginosum, 134

F

Fagerlind, F., 116, 144
Festuca:
  elatior, 67
  pratensis, 67
Florell, V. H., 88
Fragaria:
  americana, 75
  bracteata, 74, 75
  bracteata × *vesca* var. *rosea,* 74
  californica, 75
  chiloensis, 76
  collina, 75
  elatior, 75
  Helleri, 74
  mexicana, 75
  moschata, 75
  nilgerrensis, 75
  vesca, 74, 75
    var. *rosea,* 74
  virginiana, 76
  viridis, 75
Frandsen, H. N., and Ö. Winge, 94

G

Galax aphylla, 131, 151
Galeopsis:
  pubescens, 82, 126, 150
  pubescens × *speciosa,* 81
    natural hybrids, 82
  speciosa, 82, 126, 150
  Tetrahit, 81, 82, 119, 128, 150
    ecology of, 126
Galium, 152
  glaucum, 144
  Kitaibelianum, 144
  lucidum, 144
  Mollugo, 144, 151
  Mollugo × *verum:*
    experimental hybrids, 145
    natural hybrids, 145
  ochroleucum, 146
  silvaticum, 144
  verum, 144, 151
genome:
  definition of, vi
  evolution of, 152
genus, criterion for, 143
Giles, N. H., 143
Ginkgo, depletion in, 65, 66
Goodspeed, T. H., and M. V. Bradley, 73
  R. E. Clausen, and R. H. Chipman, 98
Gossypium:
  arboreum, 95
  arboreum × *herbaceum,* 95
  aridum, 95
  Armourianum, 95
  barbadense, 95
  Harknessii, 95
  herbaceum, 95
  hirsutum, 95
  hirsutum × *barbadense,* 95
  Raymondii, 95
  Stocksii, 96
  Thurberi, 95
  Thurberi × *arboreum,* 95
Graebner, P., 137
Greenleaf, W. H., 96
Gregor, J. W., 84, 121
  and F. W. Sansome, 84
Gustafsson, Å., 105, 111

H

Hagerup, O., 132, 133, 134, 135
Harland, S. C., and O. M. Atteck, 96

Haynaldia, 67
  villosa, 90
Hemizonia, 23
  Wheeleri, 67
heteroploidy, definition of, vi
Hieracium, semiheterotypic division in, 16
Hollingshead, L., 80
homologous chromosomes in interspecific hybrids:
  Aegilops speltoides × *sharonensis*, 90
  Aquilegia chrysantha × *flabellata*, 77
  Gossypium arboreum × *herbaceum*, 95
    hirsutum × *barbadense* (interspecific?), 95
  Madia gracilis × *sativa*, 22
  Nicotiana rustica × *paniculata*, 99
    glauca × *Langsdorfii*, 99
  Primula floribunda × *verticillata*, 76
Hordeum, 67
Howard, H. W., 94
Huskins, C. L., 121
hybridization, introgressive, 141

I

Ichijima, K., 74
Iris:
  setosa, 122
  versicolor, 122, 128, 149, 150
  virginica, 122, 150

J

Jenkin, T. J., 67
Jenkins, J. A., 89
Jensen, H. W., 124
Johansen, D. A., 4
Jordan, A., 83
Jörgensen, C. A., 99, 118

K

Kagawa, F., and Y. Chizaki, 87
Karpechenko, G. D., 91
Kattermann, G., 88, 138
Kihara, H., 88
  and T. Ono, vi, vii, 126
Kölreuter, J. G., 98
Kostoff, D., 96, 98, 99

L

Lactuca:
  indica, 101, 102
  indicata, 101
Lactuca—continued
  sativa, 102
  spicata, 101, 102
  spicata × *indica*, 101
Lammerts, W. E., 98
laxtonberry, 115
Layia:
  cytogenetic relationships of, 46
  nutans, 67
  pentachaeta, 2, 46
    description of, 48
    distribution of, 46, 47
  pentachaeta × *platyglossa:*
    chromosome pairing in, 55, 61
    cytology of, 53 *et seq.*
    experimental hybrid, 2, 49, 81
    natural hybrid, 61
    segregation in, 51
  pentaglossa, 2, 53, 81
    description of, 48
    fertility in, 53
    synthesis of, 49 *et seq.*
  platyglossa, 2
    description of, 48
    distribution of, 46, 48
  species, chromosome numbers of, 5
Lebedeff, V. N., 87
Levan, A., 138
Levitsky, G. A., and G. K. Benetzkaia, 87
Lidforss, B., 105, 111
Liljefors, A., 88
Lindschau, M., and E. Oehler, 87
Linnaeus, C., 86
loganberry, 112, 114
Lolium perenne, 67
Longley, A. E., 105, 134
  and G. M. Darrow, 108, 110
  and W. J. Sando, 88
Löve, Á., 123

M

Machatschki-Laurich, B., 139
Madia:
  anomala, 27
  citrigracilis, 2, 23 *et seq.*, 101, 119, 127, 128, 130, 150
    artificial vs. natural, 36, 37
    cytology of, 29
    description of, 24, 44
    distribution of, 41, 44–45
    evidence for origin of, 37, 42
    fertility in, 36

Madia—continued
  citrigracilis—continued
    synthesis of, 24
      repeated, 27
  citriodora, 2, 23, 101, 127
    description of, 24
    distribution of, 23, 41
  dissitiflora, 22
  elegans, 11, 23, 67
  gracilis, 2, 22, 101, 127, 130, 150
    chromosome races of, 38, 39, 40–42
    description of, 24
    distribution of, 40, 41
    taxonomic history of, 22
  gracilis × *citriodora*, 2, 25, 27, 101, 130
  gracilis × *sativa*, 22
  (*gracilis* × *sativa*) × *citriodora*, 28
  Hallii, 4
  nutans, 2, 4, 6
    description of, 8
    distribution of, 7
    systematic position of, 4
  nutans × *elegans*, 6
  nutans × *gracilis*, 6
  nutans × *Rammii*, 2, 6, 10, 100
    chromosome pairing in, 15
  (*nutans* × *Rammii*) × *elegans*, 11
  nutans × *yosemitana*, 6
  nutrammii, 2, 4 *et seq.*, 100, 119
    cytology of, 13
    description of, 8
    fertility in, 12
    fitness to environment, 20–21
    resynthesis of, 19
  Rammii, 2, 4
    description of, 8
    distribution of, 7
  sativa, 22, 130
  section *Anisocarpus*, 4, 6, 151
  section *Eumadia*, 6, 22
  species, chromosome numbers of, 5
  subspicata, 38, 130
    description of, 39–40, 45
    distribution of, 41, 45
Madiinae, number of genera and species in, 1
Mammoth blackberry, 115
Mangelsdorf, A. F., and E. M. East, 74, 76
Mangelsdorf, P. C., and R. G. Reeves, 67, 137
Manton, I., 139
maternals, in interspecific hybridization, 9
meiosis, degeneration of, in *Layia pentaglossa*, 58
Meister, G. K., 87
Morinaga, T., 93
multivalent association. *See under* autoploidy
Müntzing, A., 81, 86, 87, 88, 89, 116, 126, 130, 131, 138

N

Nannfeldt, J. A., 121
Navaschin, M., vii
Ness, H., 108
nessberry, 108
Newton, W. C. F., and C. Pellew, 76
Nicotiana:
  digluta, 98, 117
  glauca × *Langsdorfii*, 99
  glutinosa, 98, 117
  glutinosa × *Tabacum*, 98
  otophora, 97
  paniculata, 99
  rupa, 98
  rustica, 99
  rustica × *paniculata*, 98
  Setchellii, 97
  silvestris, 97, 117
  silvestris × *tomentosiformis*, 96
  Tabacum, 96, 98, 117, 119
  tomentosa, 97
  tomentosiformis, 97, 117
  Vaviloviana, 99
Nikolaeva, A. G., 87
Nordenskiöld, H., 84

P

Payson, E. B., 78
Peitersen, A. K., 106
Penstemon:
  azureus, 126, 150
    ssp. *angustissimus*, 130
    ssp. *parvulus*, 130
  heterophyllus, 130
  laetus, 126, 150
  neotericus, 126, 128, 150
Peto, F. H., 67
Phenomenal berry, 114
Phleum:
  alpinum, 86, 121
  nodosum, 86, 121, 150
  nodosum × *alpinum*, 84

Phleum—continued
  pratense, 84, 119, 128, 150
    ecology of, 121
  pratense × *nodosum*, 85
ploidy, definition of, vi
Plotnikova, T. V., 88
Poa:
  annua, 121, 128, 150
  annua × *supina*, natural hybrid, 122
  exilis, 121, 150
  section *Ochlopoa*, 121
  supina, 121, 150
Polunin, N., 132
polyploid complex:
  in *Galium*, 144
  in *Madia*, 37, 130
  in *Penstemon*, 130
  in *Rubus*, 108
  in *Tradescantia*, 142
polyploid series:
  in *Artemisia*, 127
  in *Cuthbertia*, 143
  in *Eragrostis*, 134
  in *Fragaria*, 75
  in *Madia*, 23
  in *Nicotiana*, 98
  in *Phleum*, 84–85
  in *Rubus*, 104, 106
  in *Rumex Acetosella* complex, 123
  in *Solanum nigrum* complex, 99
  in *Triticum* comparium, 89
  in *Vaccinium* of eastern United States, 134
  in *Vaccinium Oxycoccus* complex, 134
polyploidy, definition of, vi, 117
Poole, C. F., 79
Potentilla glandulosa, 151
Primula:
  floribunda, 76
  floribunda × *verticillata*, 76
  kewensis, 76, 116
  verticillata, 76
Prunus:
  cerasifera, 103
    ssp. *divaricata*, 103
  domestica, 103
  spinosa, 103
pseudo amphiploids, 99

Q

quasi amphiploids, 60, 80

R

Randolph, L. F., 117, 136
Raphanobrassica, 91, 93
Raphanus:
  sativus, 93
  sativus × *Brassica oleracea*, 91
Reeves, R. G., and P. C. Mangelsdorf, 67, 136
Rickett, H. W., vi
Rimpau, W., 87
Rosenberg, O., 16
Rozanova, M. A., 110
Rubus, 104 *et seq.*
  allegheniensis, 105
  arcticus, 104
  argutus, 105
  caesius, 104, 110
  canadensis, 105
  centiformis, 111
  Chamaemorus, 104, 108
  cuneifolius, 105
  deliciosus, 104
  facultative apomixis in, 105
  hispidus, 106
  idaeus, 104, 105, 110, 111, 113
  idaeus × *caesius*, experimental hybrid, 110
    natural hybrids, 111, 112
    pairing in, 112
  lasiococcus, 108
  lemurum, 107
  leucodermis, 105
  loganobaccus, 112
  loganobaccus × *parviflorus*, 115
  macropetalus, 107, 108
  maximus, 110
  neglectus, 105
  nivalis, 107, 108
  occidentalis, 105
  odoratus, 104
  parviflorus, 104, 115
  pruinosus, 111
  rusticanus, 105, 112
  saxatilis, 104
  section *Anoplobatus*, 104, 115
  section *Chamaebatus*, 107
  section *Cylactis*, 104, 108
  section *Eubatus*, 104, 105, 106, 110, 111
  section *Idaeobatus*, 104, 105, 110
  setosus, 105
  spectabilis, 105

Rubus—continued
  stellatus, 108
  strigosus, 105, 110
  strigosus × *ulmifolius rusticanus* (?), 112
  titanus, 115
  tomentosus, 105
  trivialis, 105, 110
  trivialis × *neglectus*, 109
  (*trivialis* × *neglectus*) × nessberry, 109
  trivialis × *strigosus*, 108
  ulmifolius, 105, 112
  ursinus, 106, 107, 108, 113, 114
  ursinus × *idaeus*, 113, 114
  vitifolius, 107, 108
  Warmingii, 111
Rumex:
  Acetosella, 123, 128, 150
  angiocarpus, 123, 150
  graminifolius, 123
  tenuifolius, 123, 150
Rybin, W. A., 103

S

Sageret, A., 91
Sclerocarpus, 22
Sears, E. R., 90
Secale, 67
segregation:
  in amphiploids. *See under* amphiploids
  in autoploids. *See under* autoploids
self pollination, induced in self-sterile species, 9
Sequoia, depletion in, 66
Shchavinskaya, S. A., 94
Silow, R. A., 95
Sinapis:
  alba, 94
  nigra, 93
Sitanion, 67
Skalińska, M., 77
Skirm, G. W., 130
Skovsted, A., 95
Solanum:
  luteum, 99
  luteum × *nigrum*, 99
  nigrum, 99
Spartina:
  alterniflora, 120, 150
  stricta, 120, 150
  Townsendii, 120, 128, 150
species, requirements of, 20
Stebbins, G. L., 67, 123, 153

T

Täckholm, G., vi
taxonomy, experimental method in. *See under* experimental method
Taylor, J. W., and K. S. Quisenberry, 87
Terasawa, Y., 93
Thomas, P. T., 108, 112, 113, 114, 115
Thompson, R. C., 101, 102
  T. W. Whitaker, and W. F. Kosar, 102
Thompson, W. P., 87
  E. J. Britten, and J. C. Harding, 89
Thurberia thespesioides, 96
Tischler, G., vi
Tradescantia, 151
  autotetraploids produced in, 130
  bracteata, 141
  canaliculata, 140
  canaliculata × *occidentalis*, 141
  cenospecies *virginiana*, 142
  edwardsiana, 142
  Ernestiana, 142
  hirsuticaulis, 142
  hirsutiflora, 142
  humilis, 142
  longipes, 142
  occidentalis, 140
  ozarkana, 142
  paludosa, 142
  rosea, 143
  roseolens, 142
  subacaulis, 142
  subaspera, 142
    var. *montana*, 143
  Tharpii, 142
  virginiana, 142
Tripsacum, 67
  dactyloides, 137
Triticale, 86, 119
  intercompatibility of strains, 89
  the Lebedeff strain, 87
  the Meister strain, 87
  the Rimpau strain, 87
  the Svalöf strain, 87
  the Taylor strain, 87
Triticum, 67
  comparium, 67, 88, 89
  monococcum, 90
  monococcum × *Aegilops uniaristata*, 90
  Spelta, 89, 90
  turgidum × *Aegilops speltoides*, 89
  vulgare, 89, 90

Triticum—continued
  vulgare × *Secale cereale,* 86
Turesson, G., vi, 63, 137

U

U, N., 92, 94
Upcott, M., 77

V

Vaarama, A., 104, 112
Vaccinium:
  Myrtillus, 133
  Oxycoccus, 133, 134
  uliginosum, 133, 151
    var. *alpinum,* 133
    var. *frigidum,* 133
    var. *microphyllum,* 133
  Vitis-Idaea, 133
veitchberry, 112
Viola Kitaibeliana, chromosome races of, 42

W

Webber, J. M., 95
Whitaker, T. W., and R. C. Thompson, 102
Winge, Ö., 20, 78, 82, 98, 117, 118, 131
Winkler, H., vi

Y

Yarnell, S. H., 74, 76, 105, 108, 110
youngberry, 115

Z

Zalensky, V. R., and A. V. Doroshenko, 87
Zauschneria:
  californica:
    ssp. *angustifolia,* 125
    ssp. *latifolia,* 125
    ssp. *typica,* 124
  californica angustifolia × *latifolia,* 126
  cana, 124
  septentrionalis, 124
Zea:
  Mays, 67, 136
  mexicana, 135
  perennis, 135, 151

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